Microplastic Pollution in the Clyde Sea Area:

a study using the indicator species *Nephrops norvegicus*

by

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$BSc_{(hons.)}$ MSc

Submitted in fulfilment of the requirements

for the degree of Doctor of Philosophy

October 2014

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Acknowledgements

I would like to express my gratitude to the many people who have taken the time to advise, assist, and support me during my studies; not only during my PhD, but in the in the years running up to it. I would not have been nearly as lucky without them, and to list them all would add another chapter to this volume.

Special thanks go to my family, for excusing my continuous absences at birthdays, Christmases and other "get-togethers"; my parents, Sandie and Paul Welden, for their unending patience and offers of support; and to Matthew Luckcuck, for putting up with the combined joys (and smells) of *Nephrops* dissections, benthic sediments, *R* code, and rescuing corrupted files.

Credit must also go to the former staff of the University Marine Biological Station Millport, who not only provided invaluable help and advice, but also kept me sane during my time on the island; particularly the crews of the RV's Actinia and Aora, without whom I wouldn't have a single sample. I am also indebted to my fellow researchers – particularly Darren Parker, Rosanna Boyle, Andy Watts and Amie Lusher – who were always available with advice, tips, or a friendly ear.

I was also lucky in having two encouraging and enthusiastic supervisors; Alan Taylor, whose retirement I must have thoroughly disrupted toward the end of my studies, and Phillip Cowie, who not only helped me to avoid the most common PhD student mistakes, but also didn't laugh at my attempts to find whole new ones.

Finally, I would like to thank my examiners and the convenor of my Viva for taking the time to review and critique my work.

My work was funded through the Sheina Marshall Scholarship; this, and many other PhD's would have been impossible without this kind gift.

Author's Declaration

I hereby declare that I am the sole author of the work contained within this thesis and performed all of the work presented, and that it is of my own composition. No part of this work has been submitted for any other degree.

N.A.C.Welden

March 2015

All marine species names were checked for current validity via WoRMS World Register of Marine Species www.marinespecies.org

When a name has changed the new name will appear in the text, with the named given in the cited publication in parenthesis. e.g. *Marsupenaeus (*as *Penaeus) japonicus* all names correct as of September 2014

Abstract

Microplastic pollution has been identified as an ever increasing proportion of marine litter. Despite an increase in microplastic awareness over the last decade, it represents an as yet unquantified threat to the marine environment. The relatively few studies that monitor its distribution and impact have illustrated a range of worrying effects on marine habitats and communities.

The Clyde Sea Area (CSA) is subject to many sources of terrestrial and maritime plastic input. The use of plastics in recreational and commercial vessels throughout the CSA is believed to result in large levels of microplastic fibres, which have previously been seen to be ingested by a range of marine organisms. In a study of the breakdown of commonly used polymers in benthic environments, it was found that ropes of 10 mm diameter in sub-tidal conditions release between 0.086 and 0.422g of microfibers per meter per month in the early stages of degradation. This rate would be expected to increase over subsequent months, releasing substantial amounts of fibres into the CSA environment.

In addition to the presence of numerous sources of microplastics, the CSA is relatively enclosed, and may accumulate high levels of debris as a result. Monthly sampling of the water and sediment in the CSA revealed contamination similar to that observed in other near-shore environments. Thus, it is expected that the potential threat to organisms in other areas will be similar to that observed in the CSA.

One organism known to take up microplastics is the Norway lobster, *Nephrops norvegicus*, the target of the main fishery in the CSA. In this work we examined the levels of microplastic in the gut of *N. norvegicus* from the Scottish waters. Examination of individuals from the CSA revealed both a high occurrence and high accumulation of microplastic. This was found to be much greater than in *N. norvegicus* sampled from more remote Scottish waters. As a result, *N. norvegicus* from the CSA are most likely to suffer from the negative impacts associated with microplastic ingestion than those in offshore or in areas of low anthropogenic activity.

In order to determine the potential impacts of microplastic ingestion on *N. norvegicus*, we first examined the mechanism by which *N. norvegicus* retain and egest microplastic. The position of microplastic aggregations in the foregut indicates that the gastric mill is the main obstacle to microplastic egestion. Inducing moult in microplastic-fed individuals demonstrated that expulsion of the gut lining during ecdysis enables *N. norvegicus* to reduce their plastic load, limiting plastic aggregation to the length of a single moult-cycle. In an 8 month controlled-feeding experiment retained plastic was seen to have a range of impacts on *N. norvegicus*. Feeding rate and body mass was seen to decrease in plastic loaded *N. norvegicus*, and a reduction was observed in a number of indicators of nutritional state.

The results presented in this thesis have a number of implications to the CSA and wider marine environment. The similarity in the level of microplastic observed in the CSA to that of other studies of inshore waters indicates the potential for high microplastic uptake by crustaceans in those areas. The high variability in observed microplastic abundance suggests that small-scale monitoring is unsuitable for monitoring marine microplastic debris, and that use of an indicator species may provide a more reliable method of monitoring that is not subject to small-scale heterogeneity in distribution.

The seasonal retention of microplastic by *N. norvegicus* indicates that crustaceans may provide a suitable indicator of local contamination. However, in the CSA, the high level of fibre aggregation and observed impacts of prolonged retention indicate that microplastic may be causing further pressure on an already exploited resource, reducing the stability of the valuable *N. norvegicus* population.

Abbreviations

B

BLM **Binary Linear Model**

C

CSA Clyde Sea Area

D

DDE **Dichlorodiphenyldichloroethylene**

DDT Dichlorodiphenyltrichloroethane

F

FT-IR Fourier Transformed Infrared Spectrometry

G

GLM General Linear Model

H

HCH Hexachlorocyclohexane

L

LDPE Low Density Polyethylene

M

N

NY Nylon

O

P

PCB Polychlorinated biphenyls

PE Polyethylene

PES Polyester

PP Polypropylene

S

SEM Scanning Electron Microscope

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Chapter 1 General Introduction

1.1 Overview

Records of oceanic plastic pollution date back to the 1970s (Jewett, 1976; Katsanevakis et al., 2007); more recently, the number of affected habitats and the levels of plastic debris recorded have been increasing (Barnes et al., 2009; Browne et al., 2011). With growing evidence of its negative impacts, such as entanglement of marine megafauna (Gregory, 2009) and ingestion by seabirds (Franeker et al., 2004), marine plastic pollution has become a source of increasing concern.

Although only recently recognised as an environmental issue, the production and subsequent release of plastics to the environment has been occurring for over a century. The first thermoplastics were developed in the 1860's as an alternative to diminishing ivory stocks. These first plastics were unstable and unsuitable for many applications, and mass plastic production did not begin until the Second World War when resource shortages lead to a hunt for novel materials (Barnes et al., 2009; White, 2007). Early experimentation, first with nitrocellulose to form celluloid, and later with phenol and formaldehyde to form Bakelite, led to the development of a range of plastic products (White, 2007). Since then plastic production has grown over 155 fold to 299 million tonnes in 2013 (Plastic Europe, 2014).

Despite their instabilities, even examples of the first plastics can be found in our oceans; Bakelite artefacts have been recovered from wrecks as early as that of the Titanic, sunk in 1912 (RMS Titanic, 2011). The durable nature of plastic results in debris persisting in the environment, accumulating year on year from an increasing number of sources. Plastics are ideal marine pollutants due to their buoyancy and resistance to degradation which enables debris to travel great distances before settlement.

1.2 Plastic Production

1.2.1 Raw Plastic Production

Plastic polymers are high molecular mass organic compounds, formed in a range of synthetic reactions. The main polymer structure, its backbone, is made up of covalent bonds between carbon and hydrogen atoms. Van der Waals forces cause the attraction between polymer chains. Polar and non-polar groups in parallel chains are attracted to bind chains together, however, the energy required to break these bonds is much less than that necessary to break a covalent bond (Mills, 1993).

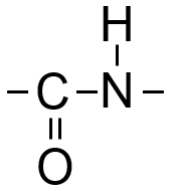
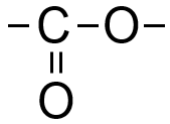
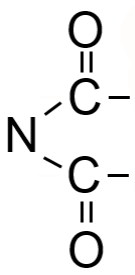
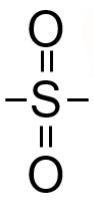
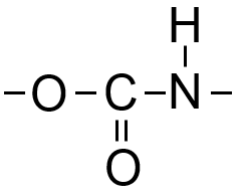
Plastics are formed as a result of polymerization reactions, which form large molecules from monomer units (McKeen, 2008). This takes place in one of two polymerization reactions; addition and condensation. By these processes resins and nibs are formed, which are the raw materials for use in the manufacturing process.

During addition polymerisation the double bond between carbon atoms within an unsaturated monomer is broken, usually via the breakdown of peroxide or another initiator molecule. This breakdown forms a free radical, which can form bonds with surrounding monomers and create new radical groups. Repetitive action of free radicals to form covalent bonds between monomers forms long chain polymers and, in the event of complete polymerization, results in no by-products (Mills, 1993).

Condensation reactions occur between monomers with two reactive groups, for example esters or amides, often resulting in the formation of a by-product, such as water. The sequential addition of further monomers to the polymer's reactive groups is known as step growth, and the resulting polymer is named according to the linking groups formed between the constituent oligomers (Mills, 1993).

Examples of the most common functional groups are shown in Table 1.1; these greatly contribute to the overall strength of the polymer backbone and the ease at which it may be degraded. Unsaturated polymers – those which contain double bonds – are more easily broken down than those with a saturated structure.

Table 1.1 The Bonds of Common Polymers

Link	Structure
Amide	
Ester	
Ether	
Imide	
Sulphone	
Urethane	

Whether formed in addition or condensation polymerisation reactions, the high molecular weight of the polymer and the strength of their bonds result in the resistance of plastics to degradation. This resistance is also determine by their molecular structure. Thermosetting plastics have a highly cross-linked structure which is only fully formed during the manufacturing process (Mills, 1993) (Figure 1.1). As a result, these plastics are less flexible and harder to break down, and it has only recently become possible to reform thermosets after curing (McKeen, 2008). Thermoplastics – such as acrylic – have long, saturated hydrocarbon chains with a high molecular mass. Unlike thermosetting plastics they can be melted and reformed into new shapes (McKeen, 2008). Their non-polar nature results in few weak sites at which the polymer can be broken down, rendering them inert, and thus they are regarded as non-biodegradable.

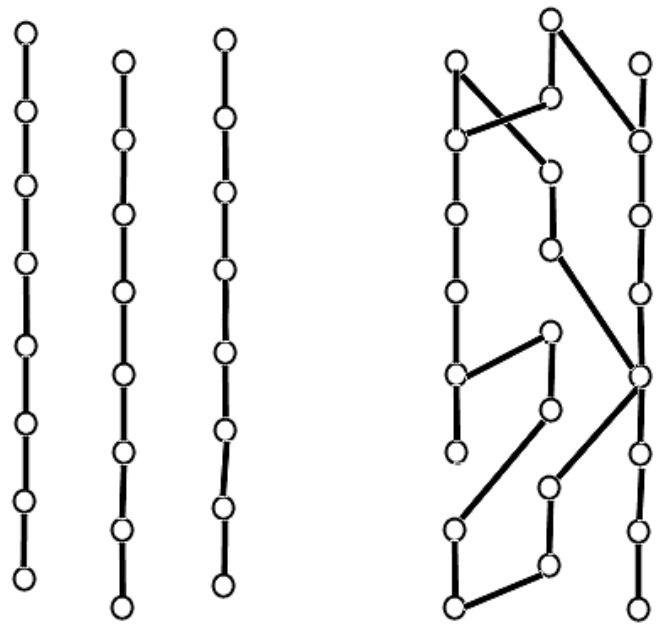


Figure 1.1 The Structure of Thermoplastics and Thermosetting Plastics

Table 1.2 The Global Distribution of Plastic Production. Source: Plastics Europe (2010)

Region	Country	Percentage Plastic Production
Europe	-	20.0%
Africa and the Middle East	-	7.3%
North American Free Trade Agreement	-	19.4%
Latin America	-	4.8%
Asia	-	16.4%
	Japan	4.4%
	China	24.8%
Commonwealth Independent States		2.9%

1.2.2 Additives

During production, a variety of chemicals may be introduced to a polymer to confer a wide range of properties (McKeen, 2008). Some additives, such as plasticizers, have been the subject of health concerns (Oehlmann et al., 2009). Small molecules are able to migrate out of a polymer by leaching, either into water or sediments or into an organism (Teuten et al., 2009), and whilst many hazardous additives have been phased out of production processes, their legacy remains in landfills and on sea beds.

Plasticizers act as emollients by holding apart polymer chains; one of the most commonly known examples are the phthalates, which are used to increase the flexibility and durability of a polymer. (Oehlmann et al., 2009). However, many have been shown to have environmental impacts and are considered hazardous due to their ease of migration from polymers (Murphy, 2001). Bisphenol A, commonly used in polycarbonates, has been seen to act as an oestrogen on a range of organisms (Feldman, 1997). Plastics can also contain heavy metals which perform various functions such as stabilizers, anti-oxidants and dyes (Murphy, 2001).

1.2.3 Modern Plastic Production and Reclamation

Global plastic production figures have risen significantly since the 1950s, when production was around 1.5 Mt annually. In 2009, 230 million tonnes of plastics were produced worldwide (Table 1.2). In 2009 50% of the plastic products produced in the EU went to waste. Of this 23.3 million tonnes, 13.1 was recovered either through recycling or incineration (GESAMP, 2010).

The UK produced 3.47 million tonnes of plastic waste in 2009. Of this only 26% or 0.9 million tonnes were recovered, leaving a pool of 2.57 million tonnes that may be released into the environment if not correctly managed (GESAMP, 2010).

1.3 Degradation

1.3.1 Abiotic Degradation Processes

Plastic polymers are durable due to their high molecular weight and non-polar structure. This reduces their susceptibility to environmental degradation (Zheng et al., 2005) and confers resistance to microbial attack (Palmisano and Pettigrew, 1992), referred to as 'recalcitrance' (Alexander, 1999). As plastics do not readily degrade, they build up in the marine environment, where they can have an impact on both human activities and marine communities. However, despite their durability, deterioration of plastics in various environments has previously been reported (Albertsson et al., 1987), although many of these reports have been attributed to microbial action upon additives, impurities and coating rather than the polymer structure.

Degradation and microbial growth has been reported to affect plastics in museum collections (Feller, 1994; Midgley et al., 2001); and a number of polymers have been identified as readily biodegradable (Alexander, 1999; Geuskens and David, 1979). Early cellulose based polymers have been seen to weaken on a time scale of 25-60 years (Derrick et al., 1993). Many of these less durable polymers, by nature or addition, contain functional groups susceptible to microbial attack, for example, amide and ester bonds (McKeen, 2008; Palmisano and Pettigrew, 1992). Ester or amide groups cause polarised regions in the polymer (Zheng et al., 2005). Polar sites are susceptible to hydrolytic cleavage, which breaks the chain down to its constituent monomers (Sudhakar et al., 2007).

A number of processes are responsible for the breakdown of polymers. The most common are hydrolysis, oxidation and thermal breakdown (Kinmonth, 1964). Degradation of polymers is caused by molecular scission, shortening polymer backbones and reducing molecular weight (Massey, 2006).

A number of factors are responsible for rate of degradation. These may be the result of the properties of the plastic; some polymers are more susceptible to attack due to weak bonds and functional groups. The introduction of additives,

such as pro-oxidants, can also increase the rate of thermal degradation (Khabbaz et al., 1999). The colour of the plastic also affects heat accumulation and thus the rate of thermal degradation (Massey, 2006).

The conditions to which the polymer is exposed also affect polymer breakdown. For example, the rate of photodegradation – the breakdown of polymers by UV light - can vary greatly with location; this is primarily due to the availability and intensity of sunlight (Statz and Doris, 1987). Photodegradation of plastics in water is much slower than in air (Andrady, 2011). In a comparison of degradation in marsh and sea water, reduced solar radiation was thought to be responsible for slower degradation under marine conditions (Breslin and Li, 1993). Decreased rates of degradation in water have been linked to biofouling and decreased temperature and oxygen levels when compared to those of air (Andrady, 2011).

Total degradation, or mineralization, of polymers results in CO_2 under aerobic conditions and CO_2 and CH_4 under anaerobic conditions (Gu et al., 1993). The extent to which mineralisation of plastics occurs is still under debate due to the time over which oxidation occurs and the ability of biota to hydrolyse susceptible bonds.

1.3.2 Biodegradation

As previously mentioned, polymers with weak functional groups are susceptible to microbial attack. Thermosetting plastics – such as polyester, Bakelite, and silicone – with their hydrolysable ester bonds (Howard, 2002), and oxidized thermoplastics are susceptible to biodegradation (Zheng et al., 2005). To enable the biodegradation of recalcitrant polymers, they must first undergo a reduction in the molecular weight by one of the abiotic processes described above (Palmisano and Pettigrew, 1992). This increases the number of weaker groups available to attack by microbes (Albertsson et al., 1987).

1.4 Sources of Marine Plastic Pollution

Plastic enters the oceans by 5 main routes; local littering, transport via the wind and currents, landfill run-off, accidental loss of fishing gear and overboard disposal (Lattin et al., 2004). The composition of debris can vary greatly depending on the scale of local land based sources, maritime activities and inputs from oceanic circulation (Gregory, 1999; Kusui and Noda, 2003). In a review of global debris studies, it was found that wind-blown plastics were the most common form of macroplastic debris (Barnes et al., 2009); however, the composition of the plastics found varies between areas.

Oceanic macroplastics mainly stem from local sources; however some plastics of terrestrial origin do become “geodrifters”. These span the globe via the great oceanic gyres. The debris from maritime activities ranges from everyday domestic items, safety gear, to industry-specific pieces such as spacers from shellfish farming and Nylon netting (Ebbesmeyer, 2009).

Studies of the composition of marine debris have shown that the fishing industry is one of the main maritime sources of marine plastic debris (Edyvane et al., 2004; Kaiser et al., 1996; Kiessling, 2003; Macfadyen, 2009). Much of this is caused by the wear and loss of ropes and nets. First introduced in the 1950s, synthetic fibres offered greater strength and durability than natural fibre ropes whilst decreasing the overall weight of the net (Valdemarsen, 2001). Since then, their use quickly became almost ubiquitous; plastics now serve numerous functions, for example, polyamides are widely utilized in the production of fishing nets and boat hulls (Sudhakar et al., 2007). In pot and creel fisheries plastic coated steel and synthetic mesh have almost completely replaced traditional materials (Galbraith et al., 2004). Due to their ability to withstand frequent and long term submersion their use spread rapidly throughout global fleets (Valdemarsen, 2001).

With such a large volume of plastics in daily use it is unsurprising that a percentage should be lost. Studies of abandoned, lost and discarded gear have shown that many kilometres of netting and countless pots enter the oceans annually (Macfadyen, 2009). Beach surveys carried out in the Falkland Islands indicate that the majority of debris originated from the fishing industry, with

42% of shore litter comprised of fishing gear (Otley and Ingham, 2003). In Oman, plastics from the fishing industry were 26.6% by abundance (Claereboudt, 2004).

Once in the oceans, nets and other debris can travel vast distances (Macfadyen, 2009). In a study of the composition of debris washed ashore at Cape Arnhem in Australia, it was found that nets originated from at least seven countries (Kiessling, 2003). These nets remain a danger to wildlife, continuing to snare both fish and other marine megafauna in a process known as 'ghost fishing' (Andrady, 1990).

1.4.1 Microplastic Pollution

A large proportion of plastic pollution has previously passed unnoticed. Microplastics, smaller than 5mm, represent an increasing proportion of plastic debris (Barnes et al., 2009). These can be broken into two subsets, "primary"; manufactured for direct use or components, and "secondary"; formed by the breakdown of macroplastics (Arthur, 2009). Primary microplastics include pre-production nibs, air-/media-blasting particles and microspheres - a constituent of many skin cleansers (Gregory, 1996). Too small for capture in wastewater treatment screens, they are passed to watercourses and subsequently the oceans (Gregory, 1996).

Secondary microplastics are formed by natural weathering and as a by-product of human activity (Figure 1.2). For example, in regions adjacent to large boat breaking yards microplastics can be frequently released into the marine environment (Reddy et al., 2006; Srinivasa Reddy et al., 2003). It has also been suggested that plastic is deliberately discarded from ships along with permitted wastes (Franeker et al., 2004).

Microplastic fragments are found on beaches globally (Srinivasa Reddy et al., 2003). On Israeli beaches, fragments were found to make up 30.6% of the plastic collected (Golik and Gertner, 1992). On the Hawaiian archipelago, fragments made up to 87% of plastics (McDermid and McMullen, 2004). Whilst weathering causes the disintegration of plastics it does not remove them from the oceanic environment (Palmisano and Pettigrew, 1992). Fragments may float alongside macroplastic debris on oceans currents and may be dispersed over vast distances.

Figure 1.1 Fragmenting Rope Recovered from Ailsa Craig

1.5 Distribution

1.5.1 Stranded Plastic Debris

Plastic has been reported in surveys of stranded shore litter on beaches worldwide. Whilst beach studies are by far the most numerous, their results are often not directly comparable due to differences in surveying and recording protocols (Cole et al., 2011). Examination of comparable surveys carried out globally indicates that the relative proportion and weight of plastic litter is highly variable (Claereboudt, 2004; Kusui and Noda, 2003; Madzena and Lasiak, 1997; Oigman-Pszczol and Creed, 2007; Storrier et al., 2007; Williams and Tudor, 2001).

Heterogeneity in the distribution of plastic may vary with geographic scale and local environmental conditions. In the Sydney area of Australia, plastic abundance reached 89.8% of items found (Cunningham and Wilson, 2003), however, in Fog Bay, Australia, abundance was only 32.2% (Whiting, 1998). However, on a smaller scale still, microplastics recovered from three sites along a hundred meter stretch of beach showed no significant variation (Dekiff et al., 2014).

This variability is thought to be due to a number of factors including scale of local land based sources, maritime activities and inputs from oceanic circulation (Gregory, 1999; Kusui and Noda, 2003). Examination of beach litter around the Sea of Japan attributed differing debris composition on Russian and Japanese beaches to local inputs (Kusui and Noda, 2003).

Debris composition can also vary seasonally, in some areas this variation has been attributed to the summer influx of tourists (Martínez-Ribes et al., 2007; Oigman-Pszczol and Creed, 2007), and recreational boating activity (Backhurst and Cole, 2000).

The scale of local anthropogenic activities is not always related to the amount of beach litter (Frost and Cullen, 1997). Plastics have been found on numerous beaches far from pollution sources (McDermid and McMullen, 2004). Due to their

shape, local currents and prevailing wind, certain beaches act as litter sinks (Galgani et al., 2000).

The distribution of plastics can vary greatly dependent on size, shape and buoyancy. Macroplastics riding on or slightly above the water's surface are subject to windage, the frictional effect of wind on the object's surface (Shaw and Mapes, 1979). Debris subject to windage can be pushed at an angle to the prevailing current affecting their resulting distribution. Studies of litter input on Tresilian Bay in South Wales have been correlated with wind speed (Williams and Tudor, 2001) and comparisons of windward and leeward beaches in Curaçao showed that windward beaches exhibited 24.2% more plastic by abundance (Debrot et al., 1999).

The effect of windage is dependent on debris shape and the area above the water's surface (Maximenko et al., 2011). Despite this, over extended timescales, convergences can be seen to herd and aggregate drifting plastic debris (Law et al., 2010). Stranded debris also undergoes re-flotation and litter undergoes cycles of burial and exhumation (Williams and Tudor, 2001). These events would reduce the effect of wind direction on plastic distribution due to changes in wind direction at re-floatation.

1.5.2 Neustonic Plastic

Not all debris is washed up along the coasts: the oceans act as a sink for vast amounts of plastic debris. Neustonic plastic, that which floats on or below the water's surface, has gained increasing publicity with the identification of the great "Garbage Patches" at the centre of ocean gyres. In the North Pacific Central Gyre, the dry mass of plastic was found at levels six times that of plankton (Moore et al., 2001). Similarly, in Santa Monica Bay plastic mass exceeded that of zooplankton collected, however, after excluding large plastic pieces the microplastic load was three times less than that of zooplankton (Lattin et al., 2004).

Neustonic plastic has the ability to cover large distances. Data from container spills and drift studies also illustrate the propensity for debris to disperse, not

only between countries but also continents (Ebbesmeyer, 2009). In a study of fishing debris stranded at Cape Arnhem, Australia, only 12% was of Australian origin. Of the remaining 88%; 7% originated from the Philippines and 72% from East Asia, 72%. The remaining 9% were of unknown origin (Kiessling, 2003).

As with beach surveys, the methodologies and reporting structures used to analyse neustonic plastic are highly variable. However, evidence indicates a rapidly growing proportion of neustonic plastic debris. Studies in the North Atlantic have shown a density of between 0.808 and 1.238 g ml^{-1} (Morét-Ferguson et al., 2010).

The first targeted survey of neustonic plastic showed that plastic distribution in the ocean is highly patchy (Shaw and Mapes, 1979). For example, the weight of plastic debris in the Kuroshio Current was found to vary by up to 18,100g per km^2 (Yamashita and Tanimura, 2007).The North Pacific is the most highly surveyed for neustonic plastic. Table 1.3 summarises the relative neustonic plastic abundance by region. The results displayed indicate large variations in the density of plastic recorded. This variation in distribution is believed to be the result of a combination of factors, and suggests that plastic will not follow predictable patterns of global circulation (Maximenko et al., 2011).

Heterogeneity in plastic distribution can also be observed vertically in the water column; this is the result of an interaction between a polymer's buoyancy and water turbulence. The density of plastics near the southern California coast was found to be highest near to the bottom and lowest in mid-water trawls. Storm events, which increased water turbulence, were found to increase the density of suspended microplastic (Lattin et al., 2004).

1.5.3 Benthic Plastic Debris

Macroplastic debris has been recorded in benthic trawls since the 1970s (Feder et al., 1978; Jewett, 1976). More recently, microplastics have been observed in sediment samples taken off Singapore, Belgium and the UK (Claessens et al., 2011; Ng and Obbard, 2006; Thompson et al., 2004). Negative buoyancy and

biofouling result in plastic collecting on the ocean floor, where it accumulates in slower-moving deep water (Galgani et al., 1996; Lobelle and Cunliffe, 2011).

Table 1.3 Density of Plastic Debris in the North Pacific Ocean. Source: 1 Yamashita and Tanimura (2007); 2, Moore et al. (2001); 3, Day et al. (1990)

Region	Density of plastic
Kuroshio Current	3600 g km^2
NP Central Gyre[1]	34.0 g km^3
California Coast[2]	2.0 g km^3
Sea of Japan[1]	128.2 g km^3
Bering Sea[3]	1.0 g km^3
Subtropical Water[3]	535.1 g km^3
Subarctic Water[3]	61.4 g km^3
Transitional Water[3]	291.6 g km^3

In areas of concentrated out in the Gulf of Lions human activity, the proportion of plastic in benthic debris can be large. Studies carried found that plastics made up to 90% of debris observed in trawls, in video recordings and in samples retrieved by submersibles (Galgani and Andral, 1998). The proportion and type of plastic can be indicative of local anthropogenic pressure, for instance, higher plastic densities have been recorded around both shipping lanes and fishing areas (Pruter, 1987). Belt transects of submerged plastic on the reefs around Curaçao have shown that 47% of debris was plastic (Nagelkerken et al., 2001).

The high proportion of plastics in samples of benthic debris suggests high volumes of plastic littering the ocean floor. In enclosed areas of the Meditterranean plastics have been seen to account for up to 95% of recovered marine litter (Ioakeimidis et al., 2014). Extrapolating from data collected in the north-western Mediterranean Sea, it has been estimated that, for a shelf area of 90,000 km^2, plastic debris would number 134.75 million items (Galgani and Andral, 1998).

1.6 Effects of Plastics in the Marine Environment

1.6.1 Financial Impacts

Marine plastic has numerous effects on both humans and the environment. Negative aesthetic impacts of plastics are recognised to result in a reduced local income from tourism (Gregory, 2009), through reduced visitors numbers and/or the cost of carrying out beach cleaning. A number of environmental issues are associated with mechanical beach cleans, including changes in the distribution of organic matter on sandy beaches (Malm et al., 2004), and alterations in community structure and decreased biomass which have been observed after only one cleaning event (Gheskiere et al., 2006).

Plastic also presents a problem to maritime activities. Macro-plastic reduces fisheries revenue through damage to nets and their contents, and increased time

spent sorting catches (Storrier et al., 2007). Debris also presents a navigational hazard including entangling propellers and fouling anchors (Macfadyen, 2009).

1.6.2 Plastics as Chemical Carriers

Marine plastics not only carry potentially damaging additives, but act as a vector for hydrophobic contaminants such as dichlorodiphenyltrichloroethane (DDT) and Polycyclic Aromatic Hydrocarbons (PAHs) which are adsorbed from the surrounding water (Frias et al., 2010; Mato et al., 2001; Teuten et al., 2009). Polychlorinated biphenyls (PCBs) have been found in plastics collected in numerous locations (Endo et al., 2005; Graham and Thompson, 2009). These contaminants represent a group of chemicals referred to as persistent organic pollutants (POPs) - organic compounds resistant to environmental degradation.

POPs previously isolated from marine microplastics are known to have a range of impacts on marine animals. DDT has been seen to bioaccumulate within the food chain, being absorbed from the water column by plankton (Cox, 1972; Rice and Sikka, 1973), and its accumulation has been observed in many marine organisms (e Silva et al., 2007; Kinter et al., 1972). The greatest impacts of DDT have been recorded in top predators, such as birds, in which the consumption of prey containing DDT has been seen to cause thing of shells and subsequent destruction of eggs (Burger and Gochfeld, 2004; Fry, 1995). PCBs are a group of organic compounds containing chlorine and a bisphenol (a pair of benzene rings). Contamination by PCBs has been observed in a variety of marine fauna (Geyer et al., 1984; Tanabe, 1988). The various PCBs differ in toxicity, but have been seen to result in high mortality even after single exposures (Kalmaz and Kalmaz, 1979). Over extended periods, they have been observed to carcinogenic effects (Kalmaz and Kalmaz, 1979). The rate at which POPs are adsorbed and leached differs between polymers (Karapanagioti and Klontza, 2007), however they can accumulate far greater levels than surrounding waters (Endo et al., 2005).

The amount of adsorbed contaminants varies geographically. Mato et al. (2000) reported variations in PCB concentration in pellets collected in four regions of Japan. Similarly, an analysis of pellets from 47 beaches around Japan found concentrations of PCBs consistent with those isolated in local mussel populations

(Endo et al., 2005). This indicates that uptake is dependent on the concentration of dissolved contaminants. The salinity of the water also impacts pollutant sorption. Previously, lower salinity was seen to result in greater sorption of DDT and phenanthrene to PVC and PE (Bakir et al., 2014).

Adsorbed pollutants become available to organisms via leaching into sediment and by the ingestion of plastic. It has been previously noted that neustonic plastics can be mistaken for prey species and directly ingested or accumulated in the food chain (Eriksson and Burton, 2003; Furness, 1985). PCBs have been shown to be transported from ingested plastics into birds (Ryan et al., 1988). Correlations between PCBs in fat tissue and plastic pellets in the gizzard of Great Shearwater, *Puffinus gravis*, indicate that uptake of pollutants is facilitated by ingestion of plastic (Ryan et al., 1988).

As debris loses buoyancy and sinks to the seabed, contaminants are "drawn down" to the benthic zones. Once in the sediment they come into contact with benthic dwelling organisms. In a study modelling the effect of adsorbed phenanthrene on the lugworm, *Arenicola marina*, it was found that relatively small initial concentrations were sufficient to significantly increase concentrations within lipid tissues (Teuten et al., 2007). These effects have since been observed in laboratory experiments examining the transfer and impact of a range of contaminants in *Arenicola*. Ingestion of PVC under laboratory conditions revealed reductions in survival, feeding rate and immune response (Browne et al., 2013). The potential for increased pollutant transfer differs between polymers, some being more effective vectors than others. The ability of a compound to migrate out of plastic is dependent on a number of factors, including polymer pore size relative to that of the additive molecule, temperature and pH (Teuten et al., 2009). The propensity for these chemicals to be passed on to an organism is the result of equilibrium between the lipids in animal tissue and that of the ingested plastic and the surrounding environment (Teuten et al., 2009).

1.6.3 Interactions between Plastic Pollution and Marine Animals

Biological impacts of plastic debris can be observed from individual to ecosystem level. Plastic pollution may result in abiotic changes to habitat which alters community structure. Microplastics in intertidal sediments have been shown to reduce the thermal conductive properties and alter drainage (Carson et al., 2011). In benthic habitats, smothering by plastic films can cause the development of anoxic conditions or reduced sunlight penetration reducing suitable habitat for colonisation (Goldberg, 1997). In soft bottomed regions plastic can form artificial hard substrata for colonization by sessile organisms, artificially elevating their numbers (Harms, 1990). Placement of marine debris on a "clean" region of sandy benthic sediment in the Saronikos Gulf, Agean Sea, showed an increase in both total abundance and number of species over that of the adjacent control; this was believed to be the result of increased settlement sites and cover (Katsanevakis et al., 2007).

Much of the available data on the biological impacts of plastic deals solely with vertebrates, many of which have been seen to be susceptible to plastic pollution. Marine megafauna such as sea turtles, Harbour Porpoise, *Phocoena phocoena*, and seals are known to ingest plastics (Baird and Hooker, 2000; Tomás et al., 2002). This may be due to their being mistaken for prey species.

Turtles are particularly vulnerable to plastic films in the water, which they may mistake for jellyfish (Tomás et al., 2002). Analysis of debris ingested by 115 stranded sea turtles revealed pelagic turtle species were more likely to consume marine litter than benthic feeding species; pelagic feeders appeared less selective then benthic feeders, with the latter showing a preference for prey which most closely resembled prey items (Schuyler et al., 2012).This accidental ingestion can result in blockage of the stomach or damage to internal organs. Fragments of plastic and latex ingested by turtles were observed to aggregate in the gut for a period of up to four months in some cases forming tangled masses (Lutz, 1990).

Vertebrates are also susceptible to entanglement in lost nets, known as "ghost-fishing" (Kaiser et al., 1996; Macfadyen, 2009). Synthetic nets take long periods to disintegrate and decaying catches attract further animals which are trapped in turn (Kaiser et al., 1996). It has recently been estimated that net losses around the UK are around 36 km $year^{-1}$ (Brown and Macfadyen, 2007). These nets

represent a significant threat to marine wildlife as they drift, and again after degradation as they join the pool of secondary microplastics.

The group most studied in relation to plastic are birds, and numerous species have been shown to ingest plastics of some form (Furness, 1983; Furness, 1985; Hays and Cormons, 1974; Pettit et al., 1981). Of sea birds, possibly the most widely recognised avian victim of this form of pollution is the Laysan Albatross, *Phoebastria immutabilis*. Plummeting population numbers were seen to coincide with large amounts of plastic being fed to chicks by parent birds (Pettit et al., 1981). This non-nutritive material has been seen to take the place of food within the stomach, resulting in starvation (Azzarello and Fleet, 1987). Wilson's Storm Petrels, *Oceanites oceanicus*, are also known to feed plastic to their chicks and other surface feeding bird species such as Fulmar, *Fulmarus glacialis*, and petrels are at particular risk of ingesting floating plastic debris (van Franeker and Bell, 1988). Evidence collected from surface feeding birds around the colonies on Foula and St Kilda in Scotland suggest that particles in flotsam are selected partially due to their size (Furness, 1985).

Plastics have also been found in shore feeding species. The Red Phalarope, *Phalaropus fulicarius*, a migrating wader which feeds on small insects and crustaceans, has been reported to consume microplastics. Analysis of body condition of recovered birds found that the proportion of body fat was negatively correlated with the level of plastic found in the stomach of the individual. This suggests that plastic in the gut prevented sufficient food uptake to lay down fat reserves (Connors and Smith, 1982). In a study of plastic ingestion and feeding in domestic chickens, *Gallus domesticus*, it was found that plastic loaded birds ate less and grew at a slower rate than control animals (Ryan, 1988). However, a study of ingested plastic by white chinned petrels, *Procellaria aequinoctialis*, indicated that there was no effect on the assimilation of nutrients and minimal damage to the digestive tract occurred (Ryan and Jackson, 1987).

Birds have also been used as indicators of changing debris composition. A study of seabirds in both the South West Indian and Atlantic oceans showed a decrease in the proportion of pre-production plastic pellets whilst no significant change in

plastic load was identified. This suggests a decrease in the proportion of plastic pellets in the survey areas (Ryan, 2008).

Plastic also finds its way into nests. Surveys of Kittiwake, *Rissa tridactyla*, showed a 17.9% increase, from 1992 to 2005, in the number of nests containing plastic items from 39.3% to 57.2% (Hartwig et al., 2007).

Fish also ingest plastic debris. In the North Pacific Central Gyre it was found that 35% of fish caught contained some form of plastic, averaging 2.1 pieces per individual (Boerger et al., 2010). Ingestion of polystyrene spherules has also been observed in juvenile flounder in the Severn Estuary (Kartar et al., 1973). Planktivorous species are particularly at risk from microplastics which resemble prey species in the water column (Hoss and Settle, 1990; Moore, 2008).

Plastics ingested by organisms at lower trophic levels, such as small fish, become available to their predators. Plastics collected from Fur Seal, *Arctocephalus spp.*, scat on Macquarie Island indicate that accumulation of plastic within the food chain occurs in a manner akin to that of pesticides, as prey species containing plastics are consumed by those at higher trophic levels (Eriksson and Burton, 2003).

Although they are much less studied than vertebrates, it has been shown that invertebrate phyla are also affected by plastic. Microplastics are available for ingestion to a range of detritivores, filter feeders and other planktivores (Browne et al., 2008; Thompson et al., 2004; Ward and Shumway, 2004). A study of filter feeding in four species of sea cucumbers found that all had ingested plastic particles (Graham and Thompson, 2009) and gut content analysis of Langoustine, *Nephrops norvegicus*, in the Clyde Sea recorded plastic in 83% of sampled individuals (Murray and Cowie, 2011). Another crustacean, *Carcinus maenas* has been shown to readily ingest microspheres, in laboratory experiments; as well as demonstrating uptake by gill structures (Watts et al., 2014b). The Blue Mussel, *Mytilus edulis*, has been shown to ingest plastics as small as 2µm. These plastic particles were observed to cross the gut wall, into the haemolymph (Browne et al., 2008). This suggests some species are capable of accumulating plastics from the marine environment within the body.

To date there has been little information collected on the biological impacts of microplastic in invertebrates. Chronic exposure to microplastics has been seen to affect survivorship in the copepod, *Tigriopus japonicus* (Lee et al. 2013), and ingestion of plastic spherules has been seen to reduce energy reserves in lugworgm, *Arenicola marina* (Wright et al., 2013). Considering the proportion of animal life represented by invertebrates, particularly in the oceans, it is surprising that there has been so little study into the effects of plastics on this group.

1.6.4 Transport of Alien Species

Plastic debris can also serve as a substrate for the dispersal of alien species. By this method sessile species, which are normally reliant on larval stages for dispersal, are able to cover greater distances as colonies attached to mobile substrate, a phenomenon also known as the Rockall Paradox (Barnes and Milner, 2005). Comparisons of communities on both natural flotsam and plastic have attributed increases of certain species such as the bryozoan, *Electra tenella*, usually found on *Sargassum* to distribution by drifting debris (Winston, 1982). The most common colonizers of plastics in Atlantic waters were found to be hydroids and bryozoans (Barnes and Milner, 2005).

Some species identified have the potential to be invasive or harmful. Toxic dinoflagellates and algal cysts colonize plastic debris, indicating that plastic may act as a vector in the spread of potentially harmful algal blooms (Masó et al., 2003). Latitude can indicate the degree to which colonization occurs, with few or no colonizers past 60° (Barnes, 2002), however, distance from land does not appear to have a significant effect on the degree of colonization (Aliani and Molcard, 2003). A number of factors limit the potential of plastics as a medium for colonization. Unlike transport by vessels, movement is passive with no guarantee of carriage to a new shore (Lewis et al., 2005) and plastic debris often supports a lower diversity than that of natural rafts (Winston, 1982).

1.7 Remediation – Further Impacts

The diffuse nature of plastic pollution means there is no single solution for controlling plastic debris. Beach cleans may remove a proportion of debris washed ashore, however as indicated previously, these can only serve small areas and have the potential to cause damage to interstitial communities. “Fishing for Plastic” schemes attempt to reduce neustonic and benthic debris by offering incentives to fishermen to dispose properly of any plastic hauled up in their nets. Woolaway’s “Points for Pounds” programme encouraged fishers to bring debris into the Kaneohe Bay pier. The scheme yielded three tonnes at a cost of US$7, 400 (Wiig, 2005). The potential to remove plastics from the marine environment is limited and the surest way to limit the impacts of marine plastic pollution is to reduce releases.

Legislation is already in place to stem the flow of plastic entering the oceans. The London Convention in 1972 banned the dumping of pollutants including solid wastes (Duncan, 1973). Dumping of plastic wastes is illegal under Annex V of the International Convention for the Prevention of Pollution from Ships. Unfortunately, plastic pollution continues: for example ‘blobs’ of melted plastic arising from incomplete incineration have been found in the South Pacific (Gregory, 1999). Sadly, in many cases there is not the will or the facilities to reduce plastic releases (NAS, 1995), and there is a limited capacity for enforcement (Maheim, 1988). Classification of potentially harmful plastics as hazardous waste may help to enable governmental environmental organisations to take a greater role in the clean-up of plastic (Rochman et al., 2013).

In addition to attempts to recover marine plastic debris, and to reduce releases into the marine environment, efforts are being made to reduce durability of plastics. “Biodegradable” polymers are currently used in many everyday products and more are under development. These contain either vulnerable chemical groups in the polymer chain or biodegradable fillers (Song et al., 2009). The method or additive used can have varying effects on a polymer’s recalcitrance. One additive commonly used to increase the rate of biodegradation is starch, however, its addition has been shown to inhibit thermal degradation of low density polyethylene films (Khabbaz et al., 1999). Degradation rates are reliant on the presence of a suitable organism and the correct abiotic conditions. Examination of bioplastic additives in estuarine

benthos identified that biodegradation by the polychaete, *Mediomastus ambiseta*, and bivalve, *Nucula annulata*, requires a ready source of nitrogen, for example ammonia (Doering et al., 1994).

1.8 Study Area

1.8.1 The Clyde Sea Area and its Catchment

The Clyde Sea Area (CSA) is a glacial estuary on the west coast of Scotland adjoining the Irish Sea (Figure. 1.3). It encompasses a volume of roughly 100 km^3, reaching depths of 180m in the deep water channels (Rippeth and Simpson, 1996; Steele et al., 1973). The catchment, covering an area of around 3,350 km^3, includes both direct terrestrial run-off and a number of rivers (Steele et al., 1973), with a combined input of between 60-700 m^3s^{-1} (Poodle, 1986). During the course of their flow the rivers pass through highly urbanized and industrialized areas, and have the potential to pick up a large debris load from numerous sources (SEPA, 2007). Due to its high population density and local industry, the CSA's main tributary, the River Clyde, has been recognized as a highly polluted river since the 1970s (Hammerton, 1986).

1.8.2 Point Sources of Plastic

Point sources of terrestrial pollution take the form of sewage outfalls and run-off from landfill sites (Figures 1.4 and 1.5). Within the CSA, sewage from numerous small communities is subject to only minimal treatment prior to discharge thus allowing a greater proportion of litter to be released than that in effluent from more urbanised areas. This debris joins plastic from oceanic sources that is carried into the CSA by the currents and local internal sources such as sewage sludge and industrial waste historically dumped near Garroch Head on the Isle of Bute (Steele et al., 1973).

1.8.3 Diffuse Sources of Plastic

Diffuse maritime sources of plastic debris include traffic from HM Naval Based Clyde at Faslane and pleasure craft from the numerous popular marinas. Litter from pleasure craft and from the many tourist beaches around the study area may constitute a significant increase in plastic input during the summer season (Gabrielides et al., 1991; Martínez-Ribes et al., 2007; Velander and Mocogni, 1998). Surveys conducted in Halifax Harbour indicated that litter from recreational sources was thought to make up 31.9% of debris collected (Ross et al., 1991).

As discussed earlier, one of the main origins of marine microplastics is the fishing industry. In 2009 the Scottish fishing fleet numbered 2,174 vessels, many operating from harbours within the Clyde Sea (O.F.N.S, 2009). In the CSA the most common fishing method is benthic trawling for *N. norvegicus* and Whitefish (Galbraith et al., 2004). In a previous study of trawling effort utilizing data loggers to record trawl maps, it was found that certain regions of the CSA are subject to higher fishing pressures than others (Marrs et al., 2002). These high intensity areas may be subject to higher inputs of fisheries related microplastic.

The number and proximity of plastic sources indicates the CSA has the potential to reach levels of plastic pollution much higher than those observed in other coastal habitats. Thus the Clyde may act as a model for plastic transport, exhibiting the characteristics of a highly polluted coastal zone.

Figure 1.2 The Clyde Sea Area, 55.7520° N, 4.9300° W

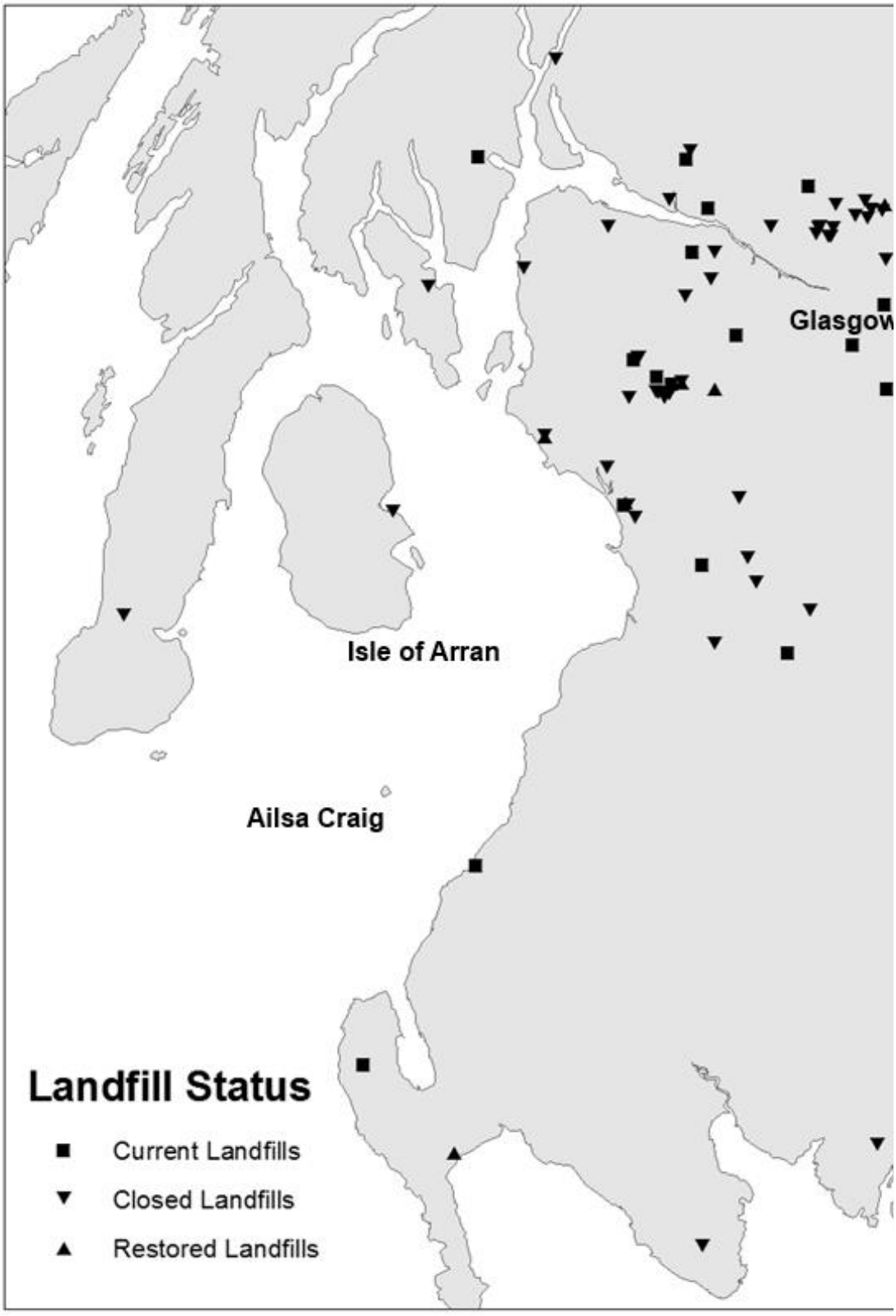


Figure 1.3 Landfills in the Clyde Sea Catchment

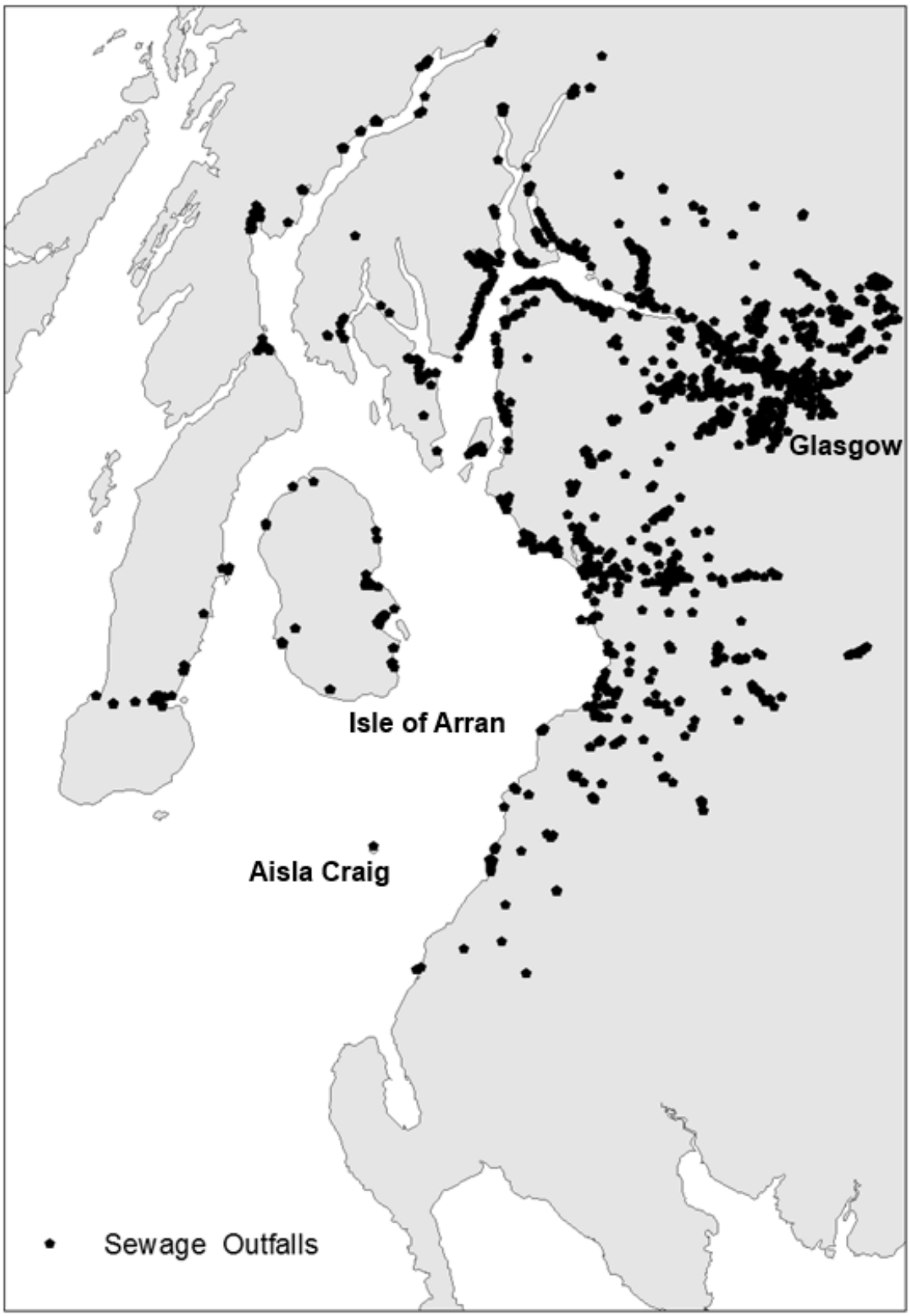


Figure 1.4 Sewage Outfalls in the Clyde Sea Catchment

1.8.4 Fate of Plastic in the CSA

Residence time and accumulation of plastic in the study area will be dependent on a number of factors; the interaction between basin flushing time, the buoyancy of plastic, and the amount of plastic being carried into the area from oceanic sources. Currents in the CSA are weak and their direction is greatly affected by wind action (Dooley, 1979). Recent calculations of the residence time of water in the CSA have produced estimates of a period of between two and four months, longer in more sheltered embayments and deeper regions (Midgley et al., 2001). This long residence time enables plastics to accumulate high levels of locally available persistent pollutants (Steele et al., 1973).

Renewal of deep water occurs only when the density of the water in the North Channel is greater than that within the Clyde basin, usually during winter months (Midgley et al., 2001). Over this period there is the potential for neustonic plastic to be encrusted and sink into benthic zones (Velander and Mocogni, 1998). The sub-tidal sediments of the CSA are mainly made up of fine grained muds in the deeper regions, giving way to muddy gravels in the shallows (Moore, 1931). In these sediments plastics, are able to settle and accumulate.

The CSA waters are highly stratified due to seasonal thermoclines, however, complete vertical mixing does occur, coinciding with a transition between summer and winter regimes (Rippeth and Simpson, 1996). Stratification has the potential to greatly affect the distribution of plastic in the water column, influencing the rates of settlement of negatively buoyant and fouled plastics.

Mixing of water between the Irish Sea and the CSA is low. This is due to the shallow sill where the sea joins the North Channel, and a front caused by the difference in thermohaline conditions which separate the homogenous North Channel from the highly stratified waters of the CSA (Kasai et al., 1999; Midgley et al., 2001). Large influxes of water from the North Channel are thought to be facilitated by favourable wind events when cross-channel winds cause significant exchange between the two water-bodies (Davies and Hall, 2000). Low water input from the Irish Sea suggest that the amount of oceanic plastics carried into the CSA would be limited, and that the majority of plastics found within the study area have originated from local sources.

The accumulation and deposition of debris in the CSA is not expected to be even. Previous studies have shown that debris is not distributed evenly in coastal environments; in certain conditions areas can act as litter sinks. In an examination of beach litter in the Firth of Forth it was found that debris retention and deposition depends on prevailing winds and local circulation, as well as flushing time (Storrier et al., 2007). Similar results were observed in the distribution of pre-production plastic pellets deposited on beaches in Canada and Bermuda (Gregory, 1983). The circulating currents in the CSA have been seen to demonstrate high spatial variability dependent on the wind direction (Davies and Hall, 2000). This suggests that – although regions favoured by prevailing wind conditions should demonstrate the highest accumulations of plastic debris – other areas may be periodically subjected to high plastic input (Figure. 1.6).

Figure 1.5 Microplastics and nurdles carried into the CSA by the 2013 Storms

1.8.4 Potential Impacts of Plastic in the CSA

As a popular local tourist area, the CSA has the potential not only to receive high levels of plastic litter, but also to suffer reduced revenues related to decreased visitor numbers as a result of the aesthetic impacts of plastic pollution on beaches. Macroplastic pollution may also affect returns from fishing activities by damaging vessels and nets.

Figure 1.6 Polymer Rope in the Nest of a Common Shag

The study area is home to numerous species known to be susceptible to plastic pollution. Harbour porpoises, *Phocoena phocoena* (Baird and Hooker, 2000), Fulmar, *Fulmarus glacialis*, Leach's petrels, *Oceanodroma leucorhoa*, Manx Shearwaters, *Puffinus puffinus* (Furness, 1985), and the Norway lobster, *N. norvegicus* (Murray and Cowie, 2011), a species of high economic importance, have already been observed to directly ingest or bioaccumulate plastics. Visual evidence indicates numerous interactions between fauna and plastic debris, for example, in visual examinations materials used at nest sites (Figure 1.7)

The number of susceptible species and varied sources of pollution suggest that plastic debris in the Clyde is able to enter the food chain at numerous trophic levels, and the potential for bioaccumulation is high. So far there have been very few studies on the effects of plastic on the lower trophic levels, particularly invertebrates. This gap in the literature concerning plastic uptake into the food chain reduces the accuracy of predictions concerning an ecosystem's ability to withstand plastic pollution.

1.9 Aims and Objectives

Once thought to be only aesthetically distasteful, the biological impact of marine plastic has only become apparent over the last two decades. Long lasting and able to traverse the vast distances of the open ocean seemingly intact, plastic is accumulating in all corners of the marine environment. To understand the impact of microplastic debris, the gaps in our understanding of uptake and assimilation of plastics from the environment must be addressed.

As wild caught *Nephrops norvegicus* have previously been observed to contain large aggregations of microplastic, they will be used as model species. By focusing on this particular species we aim expand the current knowledge of the effects of microplastic on marine invertebrates and to determine the suitability of *N. norvegicus* as an indicator of microplastic pollution. The following chapters aim to expand upon the current understanding of the accumulation and fate of microplastic pollution in marine environment.

Chapter Two aimed to determine the potential for microplastic uptake by the langoustine, *N. norvegicus* from three sites around Scotland. The amount and type of microplastic ingestion by *N. norvegicus* in the Scottish waters is determined by examining the level of microplastic contamination in the gut contents. Finally the factors responsible for any variation in microplastic in *N. norvegicus* were statistically examined for individuals sampled from the CSA.

Chapter Three aimed to establish a degradation rate for polymer ropes commonly used in the CSA. In order to achieve this, the rate of sample

fragmentation and changes in mechanical properties were observed in relation to known degradation factors.

In Chapter Four we aimed to establish a baseline for microplastic contamination in the CSA and examine the scale of monthly microplasticvariation as a result of environmental factors. To achieve this, monthly samples of sediment and floating microplastic were recovered from four sites in the Clyde Sea. The level of microplastic contamination was then compared to abiotic conditions in order to determine the factors responsible for microplastic aggregation in the CSA.

Chapter Five aimed to examine any morphological features that may impact the retention and subsequent impacts of ingested microplastic. Endocasts and SEM images were used to determine the morphology of the gut and gastric mill; this was then compared to the mass of retained microplastic in individuals of the same size.

Finally, in Chapter Six we aimed to determine the biological effects of microplastic retention. In order to predict the impact of plastic aggregations on wild crustaceans, *N. norvegicus* were exposed to diets contaminated with microplastic over a period of eight months, and the resulting body condition compared to that of individuals either starved or fed a “normal” diet.

Chapter 2 Ingested Microplastics in *N. norvegicus* from the Clyde Sea Area

2.1 Introduction

The size of marine plastic particles has been seen to decrease over recent decades. This has resulted in growing interest in the environmental impacts of marine debris (Andrady, 2011; Barnes et al., 2009). The size of microplastics enables uptake by organisms that may not otherwise be affected by plastic debris (Thompson et al., 2009). Many microplastic particles are of a similar size to planktonic organisms; as a result, microplastic ingestion has been observed in a range of invertebrate species including amphipods (Browne et al., 2008), echinoderms (Graham and Thompson, 2009) and crustaceans (Murray and Cowie, 2011).

Uptake of these plastics may occur through a number of routes. Fragmented plastic may remain in the water column for long periods before settlement. Suspended microplastics are available to suspension feeders and other planktivores, which actively ingest microplastic debris (Frias et al., 2010). Echinoderm larvae kept in plastic-seeded seawater were seen to readily take in polystyrene microspheres (Barnes et al., 2009). Similarly, the barnacle, *Semibalanus balanoides*, has been observed to take in particles over the space of a few days (Thompson et al., 2004). Subsequent observations have shown two uptake methods, via the gill microvilli, and by the movement of cilia in the stomach (von Moos et al., 2012).

There are many reports of selective ingestion of large plastic debris. This is believed to be the result of floating plastic resembling preferred prey, such as jellyfish (Furness, 1983; Ryan et al., 2009). Microplastic ingestion may also occur selectively - as small plastic fragments may resemble plankton, fish eggs and other food items in the water column; this has previously been observed in laboratory trials using freshwater cladocerans (Bern, 1990).

After losing buoyancy, secondary microplastic particles can accumulate in marine sediments. As indicated in the previous chapter, plastic fragments are

widely reported in surveys of beach debris (Debrot et al., 1999; Kusui and Noda; Madzena and Lasiak, 1997; McDermid and McMullen, 2004), and also in benthic sediments recovered from Singapore (Ng and Obbard, 2006), Belgium (Claessens et al., 2011) and around the UK (Thompson et al., 2004). The types of microplastic reported range from fragmented plastic pieces, to crumbled films and fine fibres (Thompson et al., 2004). This litter can be highly variable in both weight and composition. (Kusui and Noda, 2003).

Plastic in sediments may be ingested by a range of deposit feeders. Studies of animals kept in contaminated sediments have shown particle uptake by Lugworms, *Arenicola marina* (Thompson et al., 2004), and by deposit and filter-feeding sea-cucumbers (Graham and Thompson, 2009). Distribution of plastic in sediments is known to be highly variable (Browne et al., 2010) and animals living in areas containing high amount of plastic debris would be at greater risk of microplastic consumption.

Although ingestion of plastic by invertebrates has been recognized for almost a decade, the factors responsible for its consumption are little understood. Plastic may be picked up either passively or actively, depending on the feeding method; an individual's intake depending upon the available pool of plastic and feeding rate. The heterogeneous distribution of plastic suggests invertebrates will be at greater risk of ingesting plastic in areas of high input or high retention.

Information on the fate of ingested plastics in invertebrates is minimal, and for most species it is unclear to what extent accumulation within an organism occurs (Cole et al., 2011). The degree to which ingested plastic accumulates within an organism is governed by the recalcitrance of the polymer ingested and an individual's ability to excrete plastic particles. The variable morphology of invertebrate digestive tracts may result in a number of groups being more susceptible to plastic retention.

Lugworms have been observed to egest plastic with digested food (Bessling, 2012). However, in some species not all plastic will be expelled from the organism, and may accumulate either in the gut or the body tissues. In the Blue Mussel, *Mytilus eduilis*, ingested polystyrene has been seen to translocate into the organism's circulatory system (Browne et al., 2008).

Ingested microplastics may act in a similar way to other forms of non-degrading marine pollutants, for example heavy metals (Bryan, 1971), which cause both acute and chronic impacts (Lussier et al., 1985). Acute impacts resulting in the death of an individual, for example damage to the gut, may pass un-noticed in invertebrates in sub-littoral habitats or littoral species too small to be recorded. As such these impacts may be vastly under reported when compared to those of larger vertebrate species.

Chronic impacts are caused by the build-up of small microplastics over an extended time period. Chronic impacts may include a range of sub-lethal impacts, which may result in mortality if a critical threshold is reached. For example, chickens fed on plastic have been shown to exhibit reduced growth rates and low fat stores (Ryan, 1988).

Microplastics ingested by invertebrates also present a risk to higher trophic levels due to accumulation in the food chain (Browne et al., 2008; Thompson et al., 2009). For example, laboratory experiments with *N. norvegicus* have shown the uptake of plastic filaments seeded into fish (Murray and Cowie, 2011). In addition, shore crabs, *Carcinus maenas*, were seen to take in plastics from contaminated mussles, *Mytilus edulis*, which had previously ingested 5µm plastic microspheres (Farrell and Nelson, 2013). However, the potential for accumulation may be limited to organisms below a size threshold, above which excretion of the microplastics is possible.

2.1.1 *N. norvegicus* and Plastic

Nephrops norvegicus are decapod crustaceans found in fine sediments at depths between 20 and 800 meters across the Northeast Atlantic and in the Mediterranean. *N. norvegicus* reside in 20-30cm deep burrows dug into the sediment (Tuck et al., 1994), from which they emerge periodically to feed. This periodicity can be split into circadian (daily) and ultradian (twice daily) rhythms, dependent on depth and light penetration (Aguzzi and Sardà, 2008). An opportunistic predator, its diet is mainly composed of bivalve molluscs, polychaetes, echinoderms, fish and crustaceans including conspecifics; this live prey is located by touch and sight and occurs in shallow coastal areas where

light levels are sufficient (Aguzzi and Sardà, 2008). *N. norvegicus* also act as scavengers, using chemoreception to locate both naturally occurring carrion and by-catch from local fishing activity (Cristo and Cartes, 1998; Parslow-Williams et al., 2002).

The feeding and growth patterns of male and female *N. norvegicus* differ following the onset of sexual maturity. In winter, ovigerous females do not emerge from their burrows, seldom feeding (Aguzzi et al., 2007). The presence of eggs, carried on the pleopods, also necessitates that females do not moult during this period – limiting mature females to only one annual moult rather than two (Farmer, 1975). Feeding in *N. norvegicus* and the morphology and function of the gut is further examined in Chapter Five.

N. norvegicus are a species of high economic importance throughout both the Mediterranean and Northwest Europe (Graham and Ferro, 2004). Reported landings from fisheries in the west coast of Scotland totalled £78.3 million in 2009 (2009). Capture methods include creels and benthic trawling, with the latter being prevalent (Catchpole and Revill, 2008; Graham and Ferro, 2004). Trawl gear is mainly comprised of nets and lines of synthetic rope and is subjected to high degrees of wear.

The Clyde Sea Area (CSA) is the site of a large *N. norvegicus* fishery (2009) and is subject to high levels of maritime activity stemming from both the fishing fleet, and from traffic using the area's popular marinas. Pollution arising from these sources mix with terrestrial plastic releases from the CSA's highly industrialised catchment. The combination of sources results in a zone of high plastic contamination risk. *N. norvegicus* collected from the CSA in 2009 have previously been identified as containing high levels of plastics. Of the 120 individuals sampled, 83% were found to contain of plastic within the stomach, ranging from a few strands to a tangled ball of filaments (Murray and Cowie, 2011). Whilst the results demonstrate high levels of plastic uptake, the complex interaction of factors precludes the identification of those responsible for plastic accumulation.

2.1.2 Aims and Objectives

This chapter aims to examine the extent of plastic accumulation in *N. norvegicus* sampled from sites around Scotland. In order to examine the null hypothesis that there is no uptake of microplastics by *N. norvegicus* in Scottish waters the gut contents of animals from three locations were analysed to determine the extent of microplastic contamination.

Our second aim was to determine the factors responsible for microplastic uptake using the null hypothesis that there is no significant relationship between microplastic levels and biotic factors. This was examined using a large sample size of 1000 gut samples from *N. norvegicus* from the Clyde Sea.

2.2 Methods

2.2.1 *N. norvegicus* Collection Methods

Examination of the factors influencing the occurrence and aggregation of plastic was carried by analysing the stomach content of 1000 *N. norvegicus* collected from two sites in the CSA. Tows were taken at Skelmorlie Bank, at depths ranging from 56 to 78 metres, and in the Main Channel at depths between 69 and 110 metres in May, June and August. The catch was hand sorted into equal portions of males and females, then split into subsamples of, 20-30, 30-40 and 40-50 mm carapace length, and frozen at -5°C within 30 minutes of landing.

2.2.2 Dissection and Stomach Content Analysis

Batches of *N. norvegicus* were defrosted prior to dissection and the sex of each individual was recorded along with its carapace length (measured from eye socket to the posterior end of the carapace). The moult stage was determined by the hardness of the carapace behind the eye socket as described in Milligan (2009). Animals with a rigid carapace were recorded as “hard” and the individual regarded as being part way through the moult cycle. When the carapace could be easily compressed between thumb and forefinger, the animal was recorded as “soft” and individuals were treated as being either in pre-moult or in post-moult

when calcium in the carapace is depleted. Those animals with a paper thin, extremely soft carapace were recorded as “jelly”, having just moulted.

The stomach and hind gut were then removed (Figure 2.4), before being stored in 80% ethanol to preserve their contents prior to analysis. Following a minimum of 24 hours, the stomach contents were removed and the proportion of the stomach fullness recorded as empty, 1-25%, 26-50%, 51-75%, or 76-100% full. Hard food items in the gut content, such as mollusc shells and crustacean carapaces, were identified as fully as possible using a stereomicroscope (Parslow-Williams et al., 2002), and the presence of mud and algae was also recorded.

Plastics present in the sample were removed by hand and classified as follows - up to five strands, strands and a loose ball of filaments, a tight ball of filaments (Murray and Cowie, 2011). Other plastics present, such as films and pre-production pellets were also recorded. A Mettler MX5 balance (Mettler-Toledo international Inc., Columbus, USA) was then used to record the weight of plastic recovered from each individual to five decimal places. Prior to weighing, any algae tangled among the plastic filaments were removed and the samples air dried for 48 hours. Each sample was weighed three times and a mean taken.

Polymer verification

Identification of common plastic types was carried out by FT-IR spectrometry using a Shimadzu 8400s spectrometer. FT-IR is frequently used to confirm the identity of potential microplastic pollutants, as well as to identify the specific polymer recovered (Hidalgo-Ruz et al., 2012). The basic FTIR spectrometer consists of an infra-red light source, some form of focussing lens, and a sensor. When the IR beam contacts the sample, it excites electrons in the bonds. Different bonds in the sample’s molecular structure absorb different wavelengths within the IR beam; the remaining light is either transmitted or reflected to the waiting sensor. This remaining portion of the spectrum is then translated into a two dimensional spectrum plot.

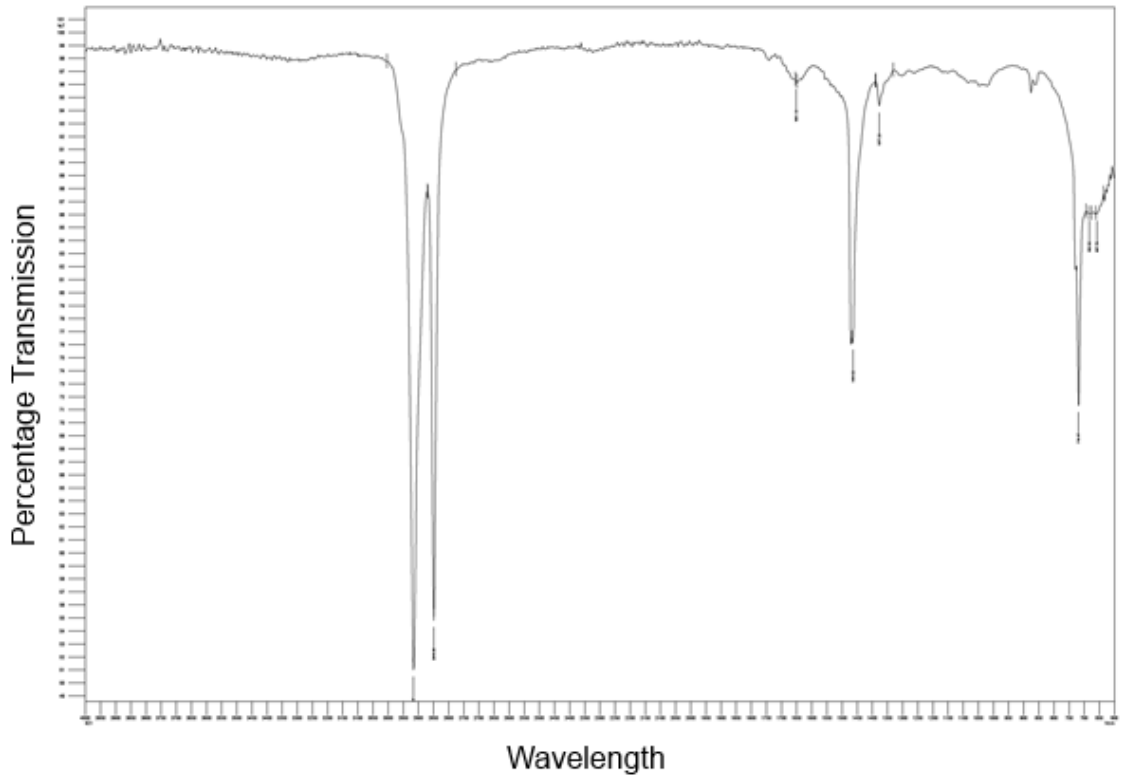


Figure 2.1 FT-IR spectrum of absorbance showing percentage transmission for polyethylene.

The peaks and troughs observed in an FTIR spectrum correspond with regions of absorbed light energy. Each material has different numbers and types of bonds, the wavelength and intensity of the absorbed light translate to the position and height of the resulting peaks; as a result, the material will give a unique spectrum. To the left hand side of the spectrum are the bonds caused by the carbon backbone of the polymer, and its characterising functional groups, the smaller absorbance readings to the right display traces of additives and other lightweight molecules and bonds. This is sometimes referred to as the "fingerprint" area (Figure 2.1).

Where necessary, samples used for FT-IR analysis were detangled from any aggregations prior to analysis to prevent multiple polymers being analysed simultaneously. Samples were then rinsed in distilled water and allowed to air

dry for 48 hours before being subjected to FT-IR analysis. To enable the identification of polymers, a library of spectra prepared from samples of commonly used plastics was used. These 'control' spectra were then compared to the spectra resulting from the analysis of the recovered sample.

2.2.3 Comparison with Other Areas

Previous work by Murray and Cowie (2011) indicated that the level of microplastic contamination observed in the gut content of CSA *N. norvegicus* was the result of intense anthropogenic activities and local urbanisation. In order to examine the impact of local pressures on microplastic uptake by *N. norvegicus*, the results of the CSA were compared to two reference samples collected from other Scottish waters, further from populated areas: the North Minch (NM) - off Stornoway, Lewis - and North Sea (NS) - off Noup Head, Orkney (Figure 2.2). At each location, langoustine were collected using 70 mm mesh otter trawls. Catches were frozen immediately on landing to prevent further digestion of food items within the gut. Dissection of individuals and enumeration of microplastic was carried out in the same manner as *N. norvegicus* sampled in the CSA.

2.2.4 Statistical Analysis

Analysis of the relationship between location, biological factors and the microplastic ingestion by Nephrops was carried out using Minitab 15. The levels of plastic contamination in *N. norvegicus* from the CSA, NS and NM were compared using a Chi^2 analysis.

Factors associated with the presence of plastic in Clyde *N. norvegicus* were determined by fitting a binary logistic model (BLM), commonly used with dependent variables of coded 0/1 (Milke and Ward, 2003), using the statistical software R, version 3.0.2. The factors analysed in relation to plastic occurrence were carapace length, sex, moult stage, trawl number, sampling site, and the presence and type of food. Due to the presence of potentially intercorrelated factors, a STEP function was included in the model; this was found to improve

the resulting fit, as observed by a reduction in the model's AIC. The significance of the results was then tested using an analysis of variance (ANOVA).

Minitab 15 was used to carry out a Kolmogrov-Smirnov analysis of the weight of plastic recovered. This returned a non-normal distribution and the data were subjected to Log^{10} transformation to normalize the data. A general linear model (GLM) was then used to determine the relationship between retained plastic and the recorded biological and environmental factors using the log transformed weight data as the response variable. A post hoc ANOVA was then used to examine the results of the GLM.

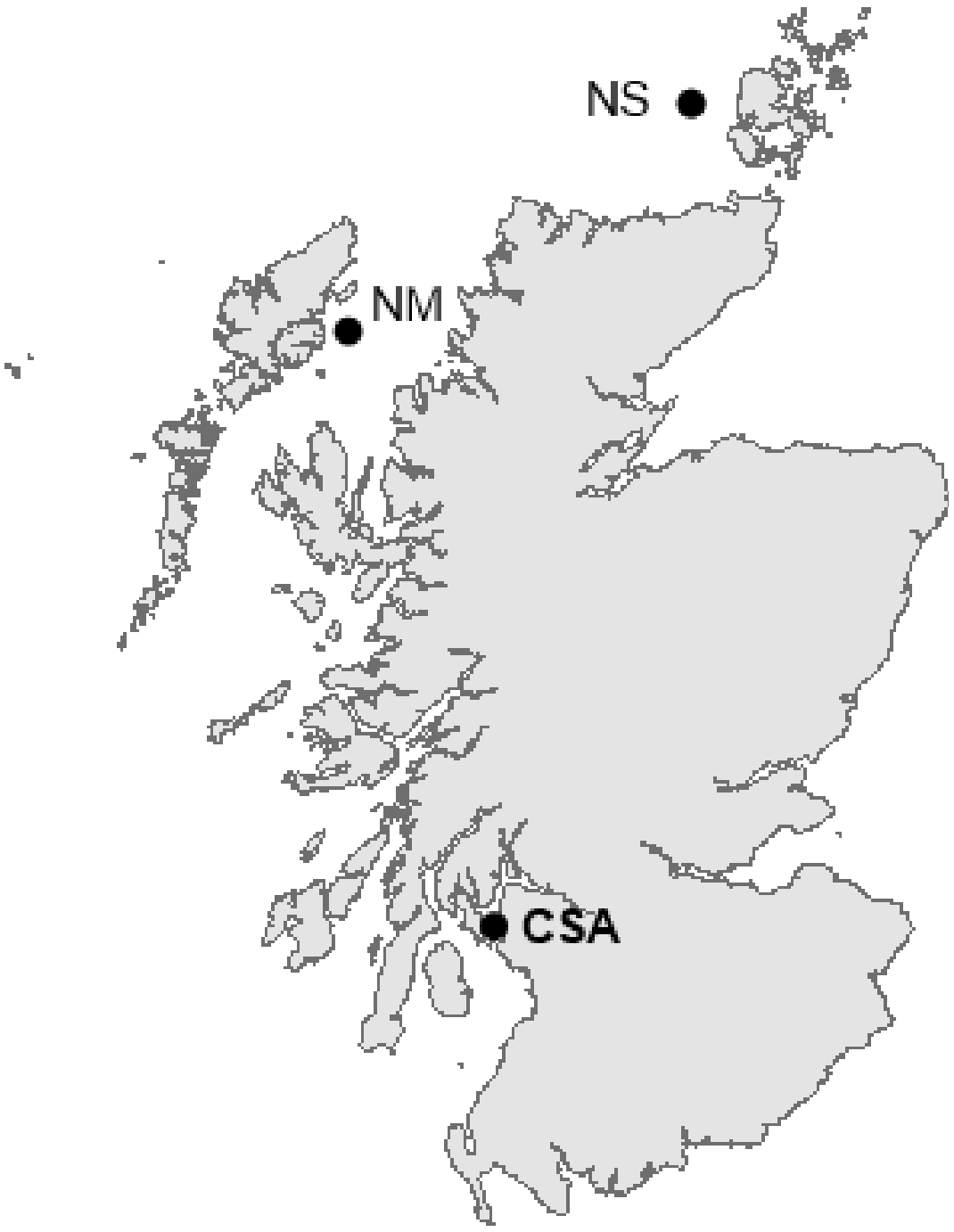


Figure 2.2 *N. norvegicus* Trawl Locations in the North Sea (NS) -3º49.07'E, 59º03.39'N, North Minch (NM) -6˚09.13'E, 58˚08.57'N, and Clyde Sea Area (CSA) -4.9751E, 55.7892N

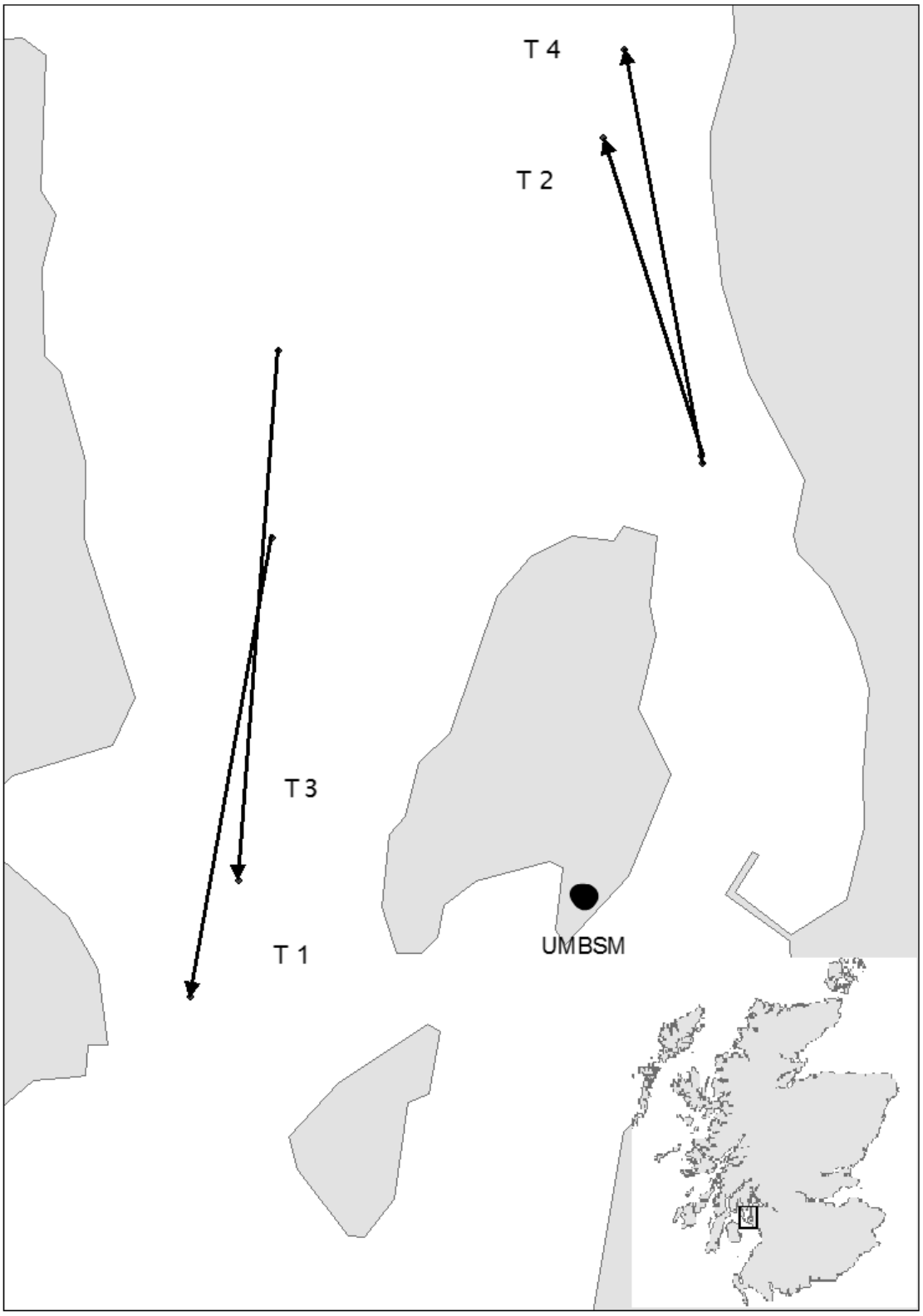
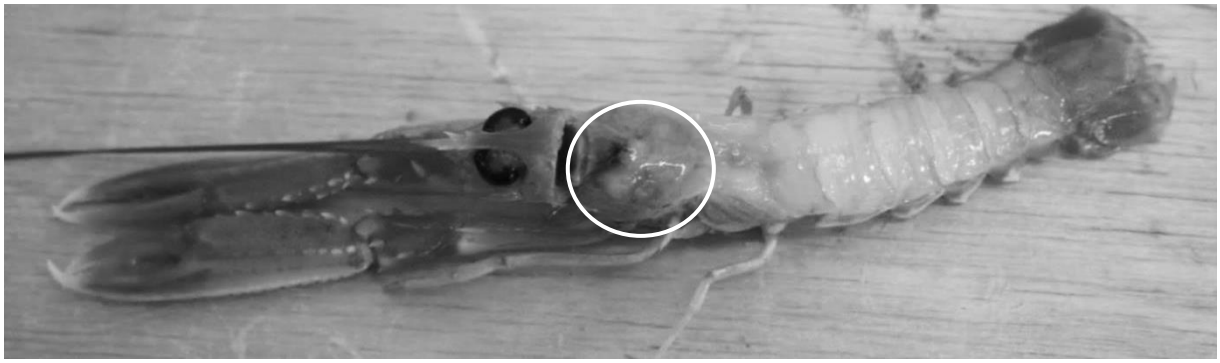


Figure 2.3 *N. norvegicus* Sampling Trawls in the CSA.
T1: 16/06/2011 -4.8903E, 55.7998N ~ -4.9093E, 55.8463N
T2: 16/06/2011 -4.9751E, 55.7892N ~ -4.9872E, 55.7368N
T3: 08/07/2011 -4.8905E, 55.8005N ~ -4.9127E, 55.8362N
T4: 11/08/2011 -4.9755E, 55.8105N ~ -4.9131E, 55.8472N



a. Individual with carapace removed showing the stomach (circled).

b. The remaining tissues are then removed and the muscle of the tail cut along the mid-line to expose the hind gut.

c. The fore gut is then lifted and cut away from mouth parts at the start of the oesophagus (circled) and the hind gut snipped before lifting out the gut intact.

Figure 2.4 Staged dissection of *N. norvegicus*: a. Removal of the carapace and somites, b. hepatopancreas removed and tail muscle divided to expose the gut, c. separation of the Stomach at the Oesophagus

2.3 Results

2.3.1 Microplastic Recovered from *N. norvegicus* in Scottish Waters

Over the course of this study 1450 *N. norvegicus* were dissected and analysed. Microplastics were found in individuals sampled at all locations; however, the number of plastic containing individuals (Figure 2.5) and the aggregation (Figure 2.6) of plastic observed varied between sites. The most common microplastic type recovered was found to be plastic fibres, but films and fragments were also observed at all sites. The mass of microplastic recovered from the North Sea and North Minch was significantly lower than that in the CSA.

Three hundred *N. norvegicus* were recovered from the North Sea sample. Of these, 91 individuals (30.4% of the sample) were found to contain plastic within the gut. 86 individuals contained plastic strands, and 5 contained plastic fragments. The weight of plastic recovered from a number of *N. norvegicus* from the North Sea was too low to accurately be recorded using the available balance. As a result, an average weight has not been calculated for this area. The maximum weight of plastic recovered from a single individual was 0.00009 g.

150 individuals were sampled from the Minch, 43 (28.7%) of which contained plastic within the gut. As in the North Sea sample, the majority of individuals seen to have ingested microplastics contained plastic fibres, and one contained a plastic fragment. The maximum weight of plastic was 0.00001 g and the average weight was 0.000005 g (+/- 0.000002 g).

A thousand *N. norvegicus* from the CSA were dissected and analysed. Of the individuals analysed, 841 (84.1%) were found to contain plastic in some form within the gut. The most commonly isolated plastics were fragmented filaments. Other plastics found were mainly films, although one pre-production nib was isolated. Fragments, films, and pellets were usually aggregated with fragmented filaments. The highest weight of plastic recorded was 0.0008 g, with a mean of

0.0004 g (+/-0.00008 g). Filaments were most commonly found aggregated into tangled "Balls" of filaments and algae (Figure 2.7), identified in 41.0% of plastic containing individuals. Chi squared analysis of the number of contaminated individuals at each site indicates a significant difference between contamination at the three locations (P value < 0.001, X^2 = 572.756, df =10). When the results were plotted on a graph the difference appears to be driven by individuals sampled from the Clyde.

2.3.2 FTIR analysis of Recovered Plastic

FTIR analysis of single microfibres proved highly laborious, occasionally resulting in unclear, 'noisy' results (Figure 2.8). Of the samples yielding sufficiently clear spectra for analysis, Nylon and polypropylene were the most frequently observed polymers. These made up 37.2%, 29.8% and 12.8% of the analysed plastic, respectively. Smaller amounts of polyethylene (mainly from ingested films) and PVC were also recovered.

By calculating the mean specific gravity for a sample made up of these polymers it was possible to calculate an approximate mean volume of 0.68mm^3 of aggregated plastic per contaminated individual. The calculated volume of the largest recorded aggregation was 9.40mm^3.

2.3.3 Factors Affecting the Presence of Plastic in the Gut of *N. Norvegicus* From The Clyde Sea

The statistical distribution of the factors affecting microplastic uptake was analysed prior to carrying out any analysis of their impact on microplastic levels recovered. The carapace length of individuals in the sample ranged from 19.8 to 59.1mm and was found to be normally distributed when examined using Kolmogrov-Smirnov analysis (P < 0.010) (Figure 2.9). The proportion of individuals at each intermoult phase differed between males and females, possibly the result of reduced moult frequency in mature females (Farmer, 1973).

The examination of identifiable prey items indicated a diet dominated by bivalve molluscs. Also frequently isolated were crustaceans, often pieces of *N. norvegicus* carapace. These two categories made up 74.1% of the identifiable gut contents with the rest being comprised of bones, presumably fish, echinoderms and polychaetes (Figure 2.10).

The results of the BLM identified moult stage, date of trawl, and carapace length as having a significant impact on the likelihood of plastic contamination in *N. norvegicus*. Recently moulted ("jelly" carapace) individuals were seen to be less likely to contain plastics than those at intermoult ("hard" carapace) ($z = -6.112$, $P<0.001$) (Figure 2.11). Carapace length was also seen to effect the likelihood of plastic presence, with smaller individuals more likely to contain microplastics ($z = -1.829$, $P<0.05$); however, the observed relationship was of lower significance than that of moult stage and trawl date.

While there was a significant difference in the occurrence and aggregation of plastic recovered from *N. norvegicus* from different geographical areas, there was no difference observed between the trawl locations within the CSA. The only non-biotic factor observed to have a significant impact on whether plastic was present within the gut was the trawl in which the animals were collected; with lower likelihood of plastic contamination in tows carried out in June; trawl three ($z = -3.675$, $P<0.001$), and August; trawl 4 ($z=4.3$, $P<0.001$) (Figure 2.12). This variation is believed to be due to a reduction in the number of recently moulted individuals later in the year.

2.3.4 Factors Affecting the Variation of Plastic Weight in *N. norvegicus* from the Clyde Sea

Log transformation of the weight of plastic recovered from the guts of CSA *N. norvegicus* resulted in a normal distribution. The results of the GLM analysis identifying the factors associated with variation in the weight of microplastic returned a similar response to that of the BLM of plastic occurrence. The results indicated that the moult stage of the individual was significantly related to the weight of plastic present ($P<0.001$), this was driven by lower weights of plastic

in recently moulted individuals (Figure 2.13). Females were seen to retain greater weights of plastic than males ($t= 4.245$, $P<0.001$).

Sampling Trawl had the highest influence over the amount of plastic retained ($P<0.001$), this was driven by a low average plastic weight recovered in trawls three and four. A significant negative relationship was also observed between the proportion of gut occupied by food and the weight of recovered plastic ($P<0.001$); individuals recorded as having no food in the foregut were observed to have the highest microplastic load (Figure 2.14).

2.4 Discussion

This study is the first to directly compare levels of microplastic uptake by invertebrates from a number of locations. From the results, it can be seen that all populations sampled showed evidence of plastic uptake. As a result it may be assumed that all *N. norvegicus* in Scottish waters are at risk of plastic uptake from the environment.

2.4.1 Plastic Recovered from *N. norvegicus* in Scottish Waters

The occurrence of plastic in *N. norvegicus* was much greater in the CSA. Here, 84.1% of individuals sampled were found to contain plastic in some form within the stomach. This percentage value supports that (83%) previously observed in a smaller study of *N. norvegicus* from the Clyde Sea Area (Murray and Cowie, 2011). This indicates that samples of around 100-200 individuals provide reliable estimates of local microplastic contamination, and are appropriate for assessing levels of microplastic contamination in *N. norvegicus* from other areas.

Although there has been a recent increase in the number of studies identifying microplastic ingestion by marine organisms, very few of these have been carried out on wild caught individuals. Gut content analysis of fish has revealed an average of 35% in the North Pacific Central Gyre (Boerger et al., 2010), and 36.5% in the English Channel (Lusher et al., 2013). Examination of gooseneck barnacles in the North Pacific Central Gyre revealed 33.5% of individuals were

contaminated (Goldstein and Goodwin, 2013). In these studies, the number of pieces of plastic are enumerated, which was not possible with the tangled filaments seen in langoustine; as a result the amount of plastic is not comparable. None the less, the percentage occurrence of microplastic in *N. norvegicus* sampled from the CSA was much higher than that observed in fish or barnacles.

Of the *N. norvegicus* sampled from the North Sea, 30.3% were found to contain plastic within the stomach, and only 28. 7% of those individuals recovered from the North Minch (NM) contained plastic, similar to those seen in fish and barnacles. The level of filament aggregation was also higher in the CSA, where the most frequently observed plastics recovered were tightly wound balls of fragmented filaments, followed by individual strands. The mean weight of the plastic balls recovered was just 0.0004 g, the volume of many of the balls found was increased by algal strands, entangled with the plastic. Of the 91 plastic-containing individuals from the North Sea, only five (6.6%) contained plastic fragments of large plastic items. No aggregations of plastic were observed and all animals contained fewer than 5 strands. The maximum weight of plastic recovered from one individual *N. norvegicus* was 0.00009 g. Similarly, in the Minch, only one individual was found to contain fragmented plastics, the rest contained plastic fibres. The largest aggregation of plastic recovered from an individual *N. norvegicus* in the NM sample weighed 0.00001; however, the mean weight was less than 0.000005 g. As mentioned in the results section, a number of the fibres recovered from NS *N. norvegicus* were below that recordable by the balance. The accuracy of the mass recorded was maintained by averaging the weight of the sample over three separate measurements.

The variation in ingested microplastic observed between the three sites indicates lower levels of environmental microplastic contamination in the Minch and North Sea. This is believed to be due to a combination of environmental and anthropogenic factors. The CSA is in closer proximity to numerous sources of microplastic, which has previously been linked to elevated levels of microplastic debris (Browne et al., 2011; Frost and Cullen, 1997; Reddy et al., 2006; Srinivasa Reddy et al., 2003).

In comparison to the other two sites, the CSA is relatively enclosed, which may result in aggregation of microplastic debris (Williams and Tudor, 2001). In the Tamar Estuary, microplastic distribution has been found to be related to wind direction, speed of local currents and polymer density (Browne et al., 2010). In the Minch and North Sea, the transport of microplastic is less constrained by benthic and coastal features. The fjord-like bathymetry of the CSA forms a number of slow current, deep water areas, which may result in high levels of microplastic deposition. The prevailing wind direction in the CSA is south-westerly, which would result in floating plastic being favourably distributed on shorelines to the north east, rather than being carried south-east to the Irish Sea. Those plastics lower in the water column would be less affected by the influence of wind direction; however, transport out of the CSA would be reduced by the raised sill at the meeting point with the North Channel.

The fibres found in these samples have numerous possible sources, and were a mix of colours and thicknesses, suggesting multiple sources. Some of these sources are terrestrial, for example, filaments may be released through sewage outfalls as a result of machine washing clothes (Andrady, 2011). Visually, many of the filaments recovered closely resembled the ropes from which many nets and ropes are comprised, and are the same blue and orange colour; however, there were also mixtures of fibres of multiple colours.

FT-IR analysis of potential microplastic was carried out using a Shimadzu 8400s spectrometer. Whilst the method provided good results for samples over 500 µm, this technique was slow for plastics below that due to the time required to position the sample to get a significantly high level of absorbance to provide a useable spectrum. Despite this, it was possible to analyse plastics from 10 % of the individuals found to contain plastic. Whilst many of the resulting spectra exhibited a degree of noise in the “fingerprint” region, preventing identification of the polymer source, it was possible to identify the type of polymer.

The results revealed high proportions of polyester and Nylon and polypropylene, commonly used in the manufacture of clothing, ropes and nets. Other plastics found were mainly films, sufficiently weathered to prevent visual identification of their origin. However, FT-IR analysis revealed them to be composed of

polyethylene. Plastic films were isolated in just 4.9% of the sampled population and their effect may be considered significantly less than that of filaments.

2.4.2 Local Environmental Factors and Plastic Ingestion by CSA *N. norvegicus*

Individuals sampled from the CSA showed significantly higher occurrence and weight of plastic within the gut than that recorded in individuals from the NM and NS. This is believed to be the result of the proximity of the CSA to anthropogenic plastic sources in the Clyde catchment. These sources have a high influence over the rate at which debris is accumulated in both the CSA basin and by resident *N. norvegicus*.

On a small scale, differences observed in both the occurrence and weight of ingested plastic between trawls within the CSA is likely to be the result of small scale heterogeneity in the distribution of plastic. This small-scale distribution is governed by a number of factors including the friction effect of wind (Shaw and Mapes, 1979), the polymer's buoyancy, water turbulence (Lattin et al., 2004), and the speed of local currents (Galgani et al., 1996). Thus, certain areas of unfavourable conditions may result in pockets of high microplastic density, particularly in regions of high input.

In addition, being a potential source of microplastic, local fishing activity may also affect distribution of plastic in the CSA. Frequent trawls would agitate the sediment and may re-suspend microplastic debris. Frequent re-suspension would reduce the impact of changeable environmental factors; those particles deposited during atypical wind states or tidal conditions would be redistributed after successive exposure to the influence of prevailing conditions.

No significant relationship was observed between the type of prey consumed and the presence of plastic. However, plastic may remain in the gut for periods long after any prey animal which carried it is digested – this is particularly true of soft bodied prey such as polychaetes (Parslow-Williams et al., 2002). Stomach content in *N. norvegicus* may be fully digested within 12-14 hours (Cristo, 2001;

Sardà and Valladares, 1990), as such, determining plastic origin in this way is unsuitable.

The most commonly identified prey types were found to be molluscs and crustaceans, as previously reported by Parslow-Williams et al. (2002). In order to identify a dietary source of plastic in *N. norvegicus*, analysis of plastic contamination in these groups at the sample sites is required. If plastic is accumulated from contaminated prey, a high proportion of contaminated individuals would be expected in these species; however, at this time it is not possible to determine whether plastic is aggregated through food or contact with contaminated sediment.

2.4.3 Individual Factors and Plastic Ingestion by CSA *N. norvegicus*

Whilst the uptake of plastic is dependent upon the level of local contamination, the weight of plastic retained is also determined by an organism's ability to egest microplastics. The negative relationship observed between carapace length and plastic indicates that plastic is more prevalent in small individuals Murray and Cowie (2011). Were plastics retained throughout an animal's life span the result would show a positive correlation; this is not the case, suggesting that *N. norvegicus* are able to egest plastics.

Previous studies have shown a relationship between *N. norvegicus* body size and weight of food consumed of approximately 0.025g of food per gram of body mass (Sardà and Valladares, 1990). Thus it may be expected that larger *N. norvegicus* will consume more than smaller ones (although this relationship may not be linear). If the level of plastic is related only to the food consumed, it might then be expected that larger individuals would be more likely to contain plastic; instead, higher likelihood of plastic was observed in smaller individuals. The low levels of aggregated plastic observed in larger individuals suggest that they are more able to egest plastics. In the following chapter, we examine the change in the morphology of the gut with increasing body size to determine the factors responsible for this variability.

High masses of plastic observed in *N. norvegicus* with empty stomachs suggest that plastics are not regularly excreted with indigestible food items. Of the 1000 *N. norvegicus* dissected, only two individuals were seen to contain plastic in their hind gut. This is supported by previous observations by Murray and Cowie (2011). Retention of plastic is believed to be a result of the structure of the gut. The anterior portion of *N. norvegicus* stomachs consist of a collection of hard arches called ossicles, which form the gastric mill. The gastric mill serves to masticate food items; however, they may present a barrier to filamentous plastics. The role of the gastric mill in plastic aggregation is explored in the following chapter.

Examining the results of both statistical analyses it is more likely that plastic is lost during the moult. During this period the carapace and the stomach lining is shed (Farmer, 1973). It follows that plastic is lost with these internal structures during ecdysis. This was observable in the reduced likelihood of plastic presence, and lower average weight of plastic in recently moulted individuals. Moult in *N. norvegicus* occurs twice yearly in males and immature individuals, and once a year in ovigerous females (Castro, 1992). The reduced rate of moult in mature females would result in higher plastic loads (observed individuals sampled from the CSA), putting them at greater risk of any negative impacts.

2.4.5 Impact of Plastic Ingestion

It is clear from the data presented here that *N. norvegicus* can retain plastics within the stomach over long periods. The negative relationship observed between weight of plastic and stomach fullness may indicate a reduced feeding rate in individuals with high plastic loads. This is indicative of false satiation, as plastic takes the place of food in the gut, previously described in seabirds (Ryan, 1988) and turtles (Lutz, 1990; McCauley and Bjorndal, 1999). Plastic in the gut also causes nutrient dilution, preventing the assimilation of ingested foods (McCauley and Bjorndal, 1999). This has previously been observed to cause reduced growth in bird species (Connors and Smith, 1982). In invertebrates, plastic ingestion has been seen to negatively affect feeding rate and body mass in the lugworm, *Arenicola marina* (Besseling et al., 2012). The potential for

negative physiological impacts related to plastic ingestion is examined and discussed in greater detail in Chapter Six.

Using the relative proportions of the polymers recovered from the *N. norvegicus* gut, it was possible to calculate an approximate specific gravity (density) for ingested microplastics. This was then converted to volume using the mean volume of plastic debris, resulting in a figure of approximately 0.68 mm^3 of aggregated plastic per contaminated individual. Whilst this may appear low, entanglement of ingestible items will cause the formation of much larger aggregations.

Whilst *N. norvegicus* have previously been seen to be highly tolerant to starvation (Mente, 2010), reduction in feeding or nutrient uptake will have a negative impact on growth rate. Links between growth rate, body mass and fecundity have been observed in a number of crustacean species (Beyers and Goosen, 1987; Hines, 1991; Lizárraga-Cubedo et al., 2003), including in *N. norvegicus* in the Mediterranean (Abellô et al., 1982). However, further work is required to establish its long term effects in *N. norvegicus*.

As well as potential nutritional effects and damage to the gut, plastics are known to carry hydrophobic contaminants and increase their draw-down to marine sediments (Teuten et al., 2007; Teuten et al., 2009). An investigation into the uptake of PCBs from polystyrene in *Arenicola marina* has shown that polystyrene has a limited effectiveness as a vector for adsorbed pollutants; however, polystyrene may still influence POP transport in the environment (Besseling et al., 2012). Until recently, the polymers used in contaminant transfer studies were selected due to their availability from suppliers; as a result, the rate of partitioning may vary substantially to that observed in laboratory trials using surrogate polymers.

In the CSA, plastics have the potential to adsorb pollutants both from sea water and from discharge waters of the catchment (Andrady, 2011). These would then become available to *N. norvegicus* through direct ingestion of the plastics themselves, and through contact with contaminated sediments and prey species which inhabit them. Uptake of hydrophobic contaminants is known to be

affected by the level of fat in tissues, for example, zebra mussel, *Dreissena polymorpha*, with higher lipid level have been seen to more readily accumulate PCBs and PAHs. *N. norvegicus*, which have comparatively low proportions of lipid in their tissues (Watts et al., 2014a), will be less prone to contaminant uptake. The lack of available information on the uptake of hydrophobic contaminants from ingested plastic and their potential as a vector highlights the need for a greater research in this area (Gouin et al., 2011).

Negative impacts may also be passed up the trophic levels. Predation upon *N. norvegicus* may lead to bioaccumulation in the food chain. Cod, *Gadus morhua*, are known to feed on *N. norvegicus* where preferred prey is unavailable (Björnsson and Dombaxe, 2004; Chapman, 1980), as well as benthic scavenging invertebrates such as cephalopods (Coll et al., 2006). Animals routinely feeding on organisms with high plastic loads may be subjected to increased plastic intake and/or the uptake of hydrophobic contaminants which are magnified in the manner of other persistent organic pollutants.

Assessing the scale of anthropogenic impacts on deep water species is challenging, due to their relative inaccessibility and the necessity for expensive equipment. As a result, increased mortality due to the ingestion of microplastic may not be recognised until exceeding the critical stress threshold; the consequence of this would be observable in a local population decline. It is essential that more is done to expose the impacts of microplastic in benthic habitats, allowing suitable management actions to be taken.

2.5 Summary

The *N. norvegicus* sampled from the CSA demonstrated a higher occurrence of plastic within the stomach compared to those from the NM and NS. Over 95% of the plastic recovered was made up of fragmented filaments which formed large aggregations in the stomach.

With increasing global microplastic levels, invertebrate populations previously only at low risk of microplastic uptake may show increased levels of ingestion. This may result in a situation similar to that observed in the CSA

These aggregations have the potential to take the place of food and to reduce feeding rate and nutritional uptake. Whilst *N. norvegicus* may go for long periods without food, large plastic loads may result in decreases in growth rate.

The potential physical impacts of microplastic ingestion, such as reduced feeding and nutrient dilution, may reduce population stability; in turn reducing the resilience to other anthropogenic activities, including fishing. Plastic releases from maritime activities including the high-wear gear used for trawling may be inadvertently destabilising the local population of their target species.

The differences observed between trawls indicate variation in the distribution of microplastic in the CSA. If this variation is found to closely replicate that present in surrounding sediments the *N. norvegicus* may be considered as a suitable indicator of microplastic pollution.

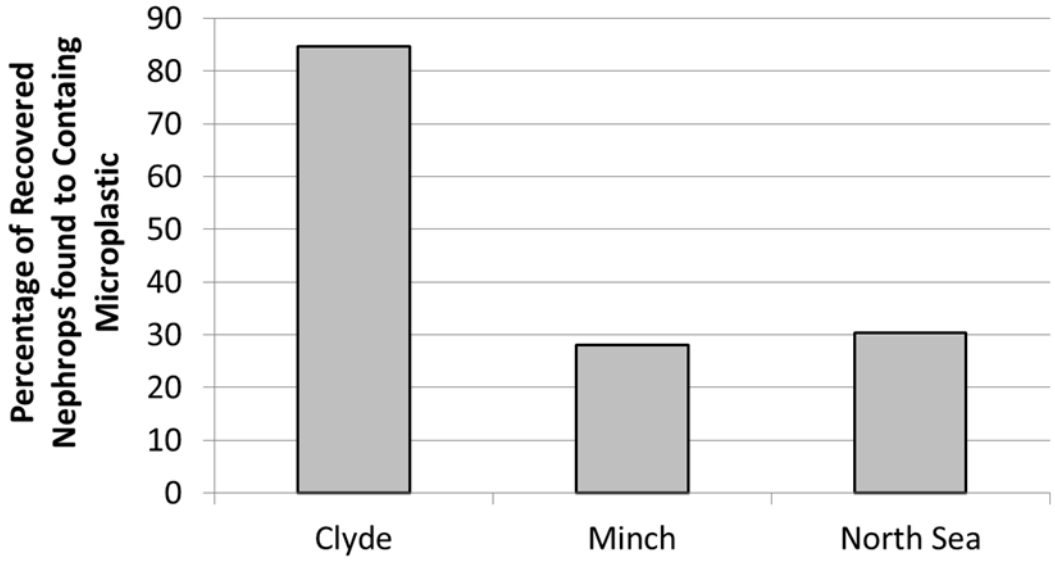


Figure 2.5 Percentage of *N. norvegicus* sampled from the CSA, NM and NS found to contain microplastic

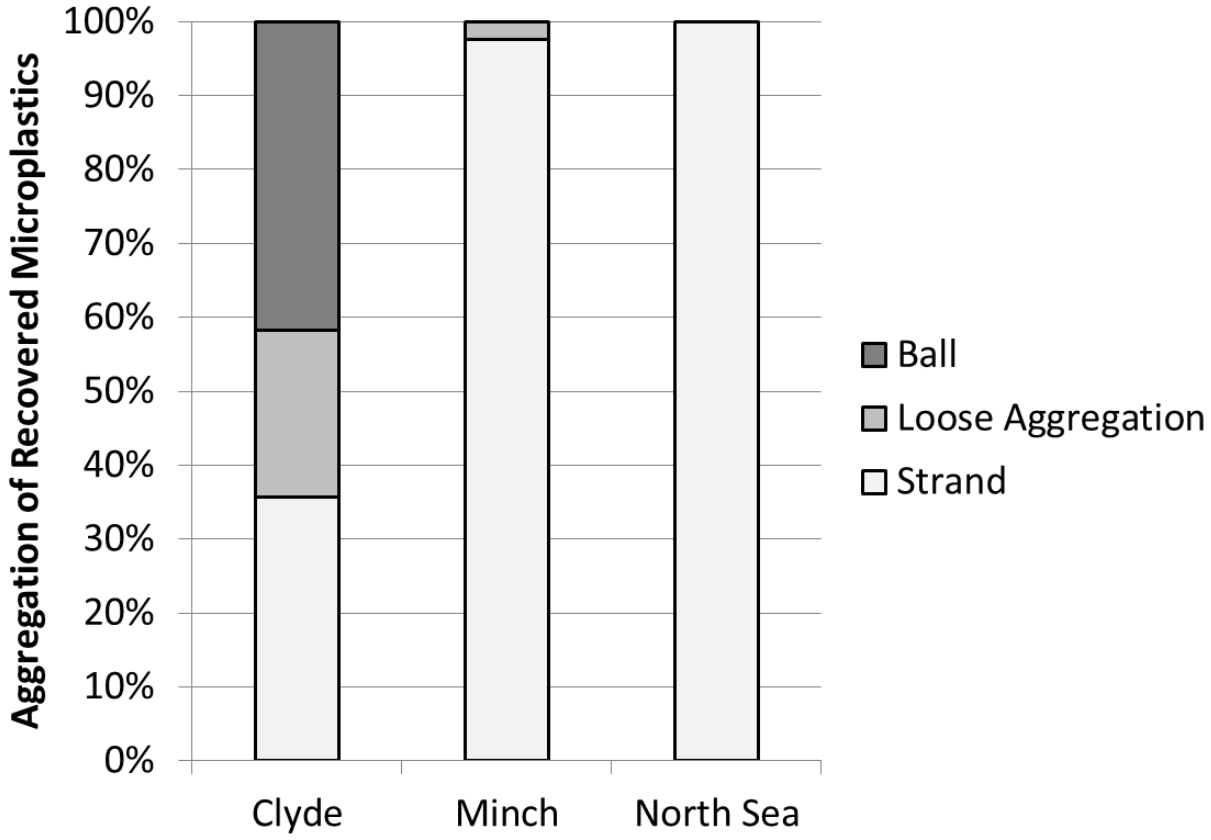


Figure 2.6 Aggregation of fibres in *N. norvegicus* found to have ingested microplastics at each of the three sampling sites.

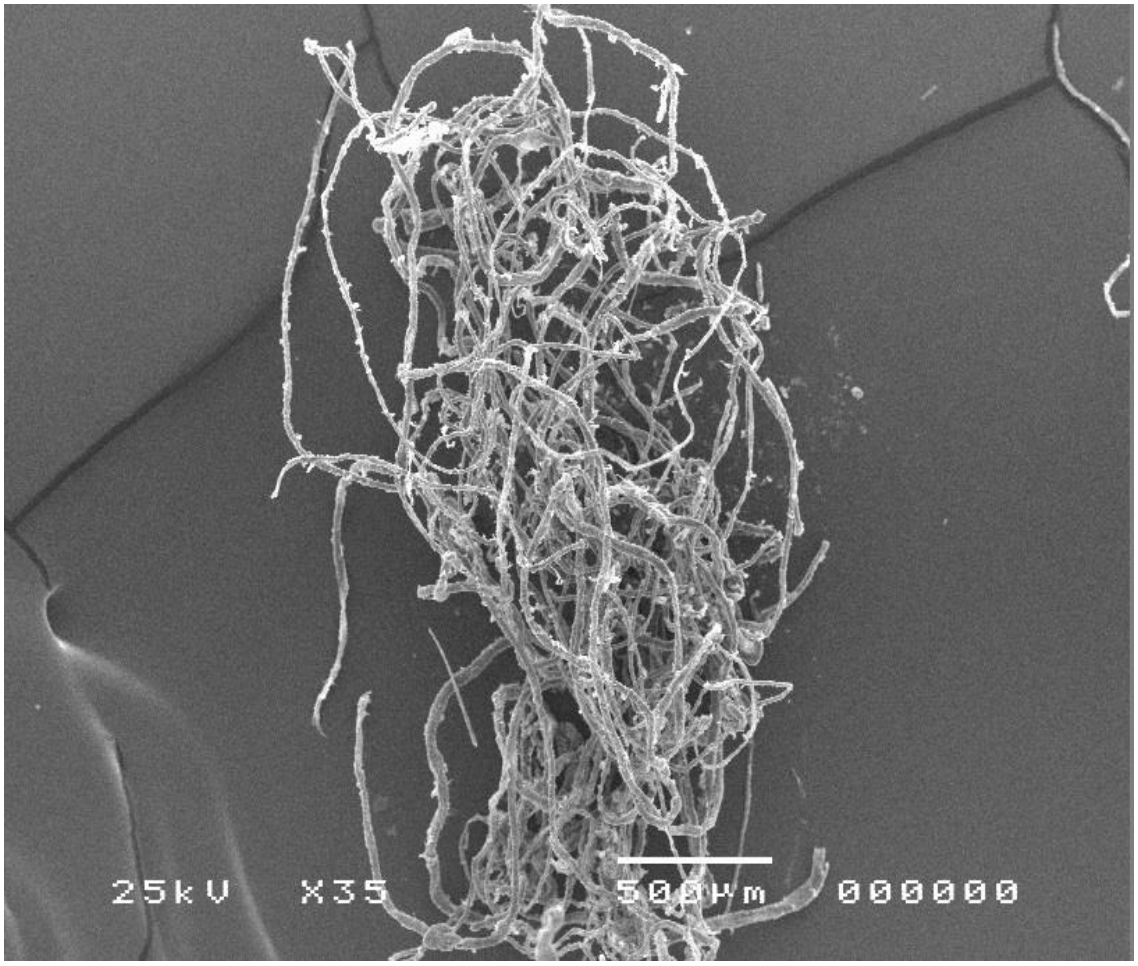


Figure 2.7 Scanning Electron Microscope Image of a Fibre "Ball" Recovered from the Gut of individual *N. norvegicus* from the CSA. The Aggregation measures approximately 3 mm by 1.5 mm (500 µm scale bar shown)

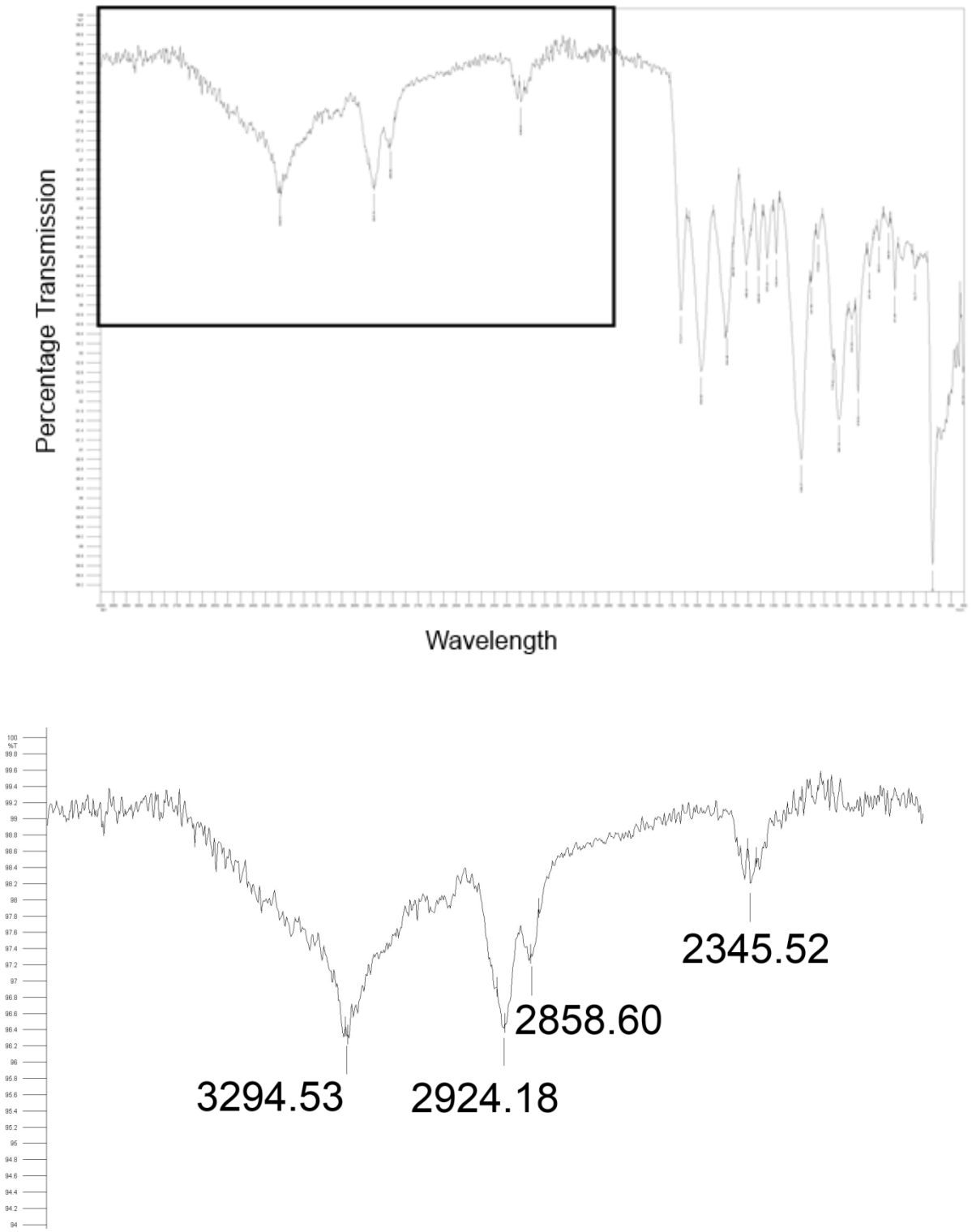


Figure 2.8 Spectrum of sample 941 – Nylon. The enlarged section shows percentage absorbance of bonds in the polymer backbone.

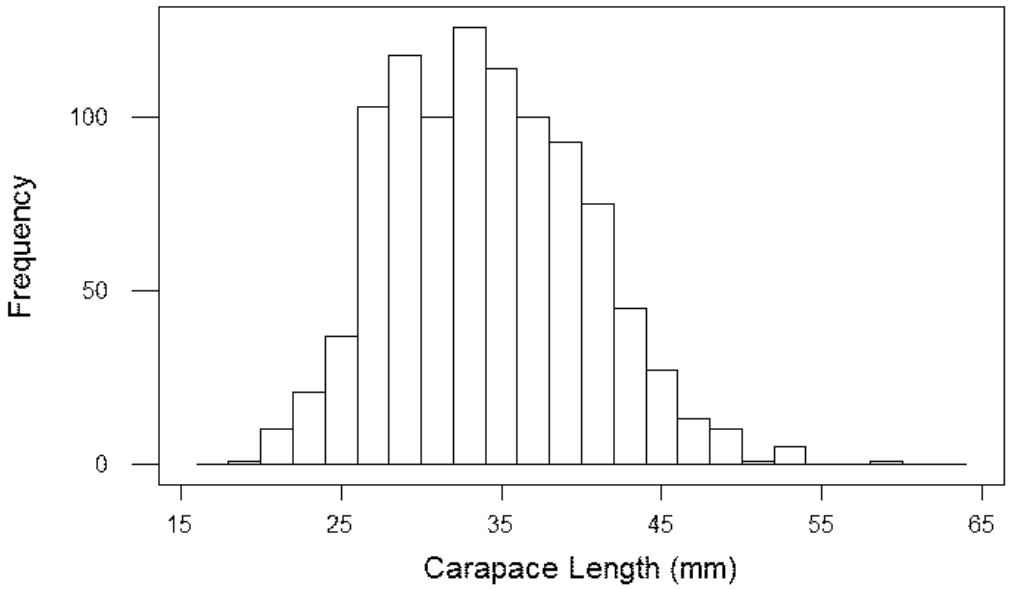


Figure 2.9 Distribution in the carapace lengths of *N. norvegicus* collected from the Clyde Sea Area.

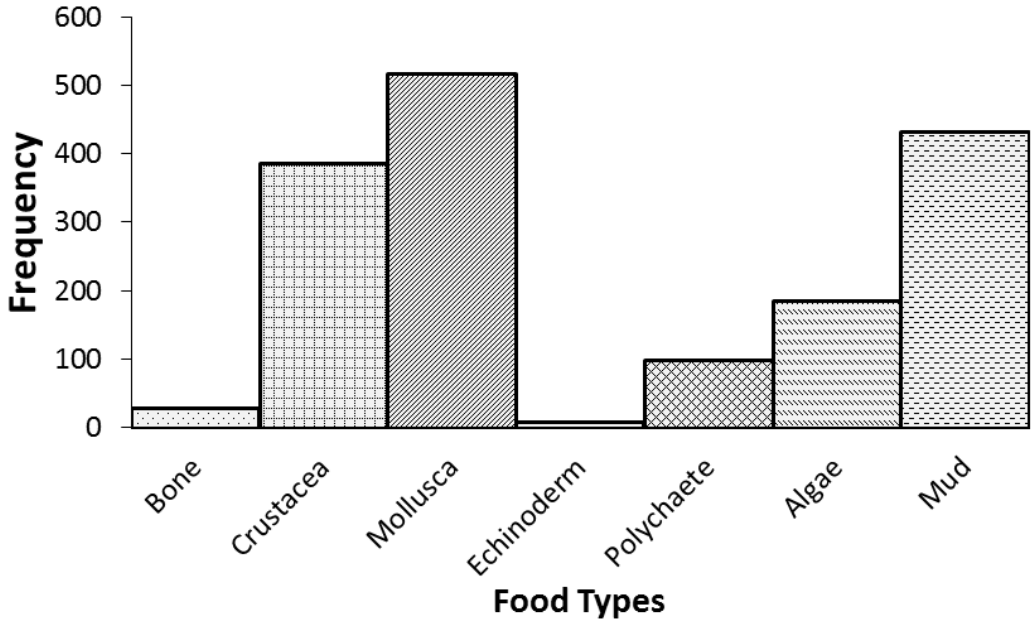


Figure 2.10 Occurrence of Identifiable Food Items in the Stomach of CSA *N. norvegicus*

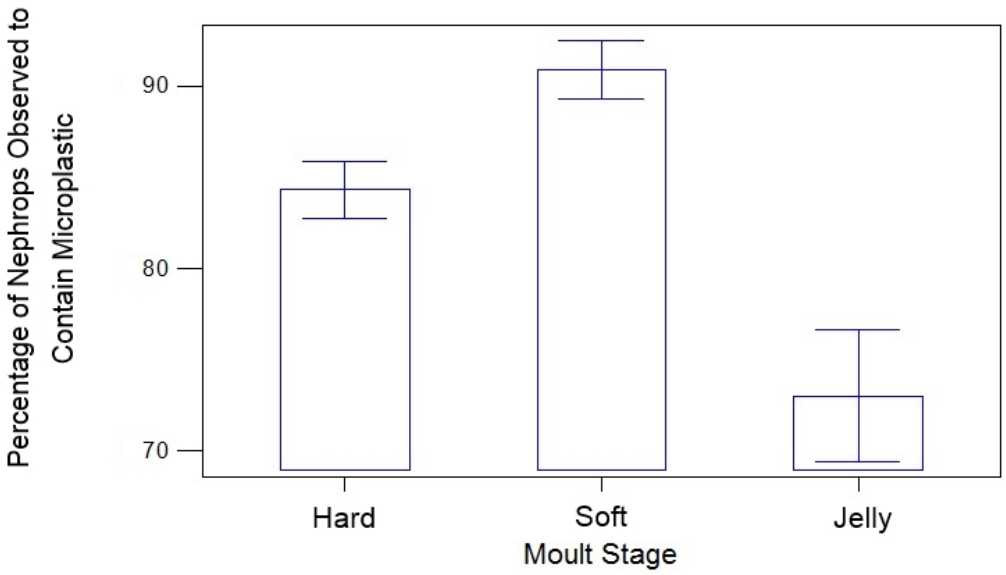


Figure 2.11 Proportion of individuals at each moult stage found to contain plastic Bars display standard deviation

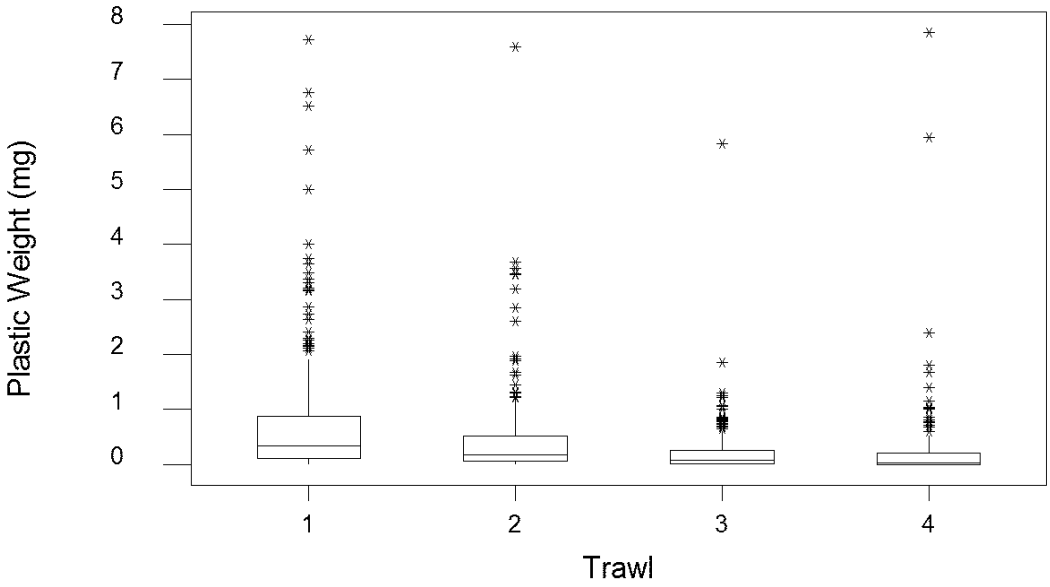


Figure 2.12 Mean Weight of Plastic Recovered from *N. norvegicus* in each trawl in the Clyde Sea Area. Bars display standard deviation, outliers are marked with asterisks *

Figure 2.13 Weight of Plastic Recovered from *N. norvegicus* from the Clyde Sea Area at Each Moult Stage. Bars display standard deviation

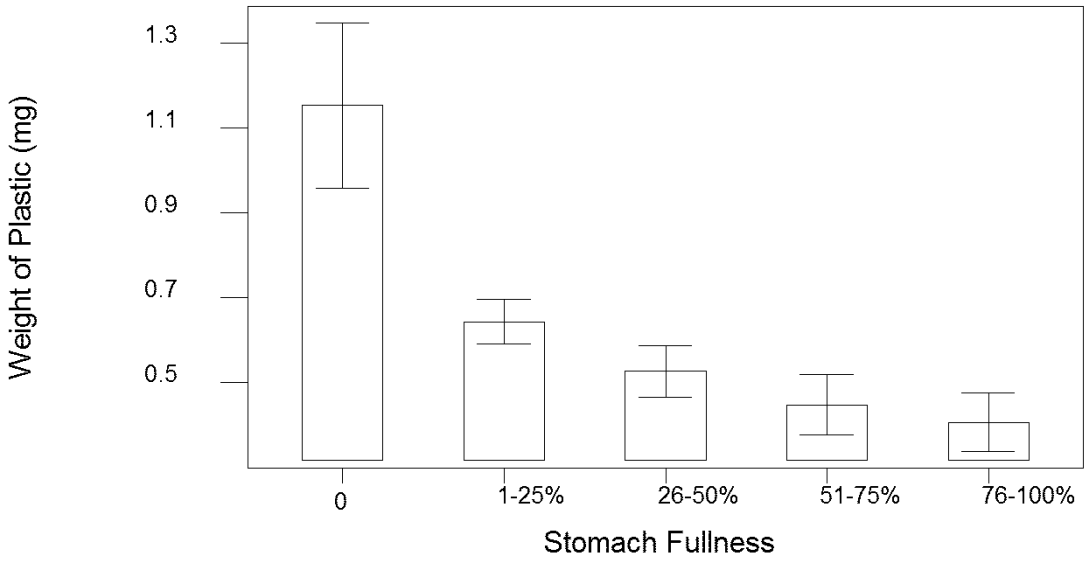


Figure 2.14 Mean weight of plastic recorded in *N. norvegicus* of increasing stomach fullness from the Clyde Sea Area. Bars display standard deviation

Chapter 3 Breakdown of Plastics in the Marine Environment

3.1 Plastic Pollution in the Clyde Sea Area

The success of plastic as a pollutant of the marine environment is primarily the result of the longevity of polymers. As outlined in Chapter One, plastics consist of recalcitrant polymers which take long periods to degrade (Barnes et al., 2009); however, plastic debris may be broken down in the environment in to numerous smaller fragments. The rate of fragmentation alters over time as a result of reduction in the mechanical properties of the material. For example, plastic films exposed to environmental conditions at a depth of 0.6m over 40 weeks showed reduction in tensile strength, and reduction in light transmittance (O'Brine and Thompson, 2010). Reduction in the mechanical properties of the polymer has a great impact on the ease with which microplastics are formed by mechanical action, such as abrasion by substrate, or the action of waves or fauna.

While much of the secondary microplastic observed in the marine environment originates from the breakdown of marine litter, there are also many items routinely used in the marine environment which are comprised of plastic polymers also subject to a range of weathering factors. This weathering results in the release of secondary microplastic fragments. Therefore, plastics in frequent use in the marine environment may also be responsible for substantial microplastic releases. FT-IR spectra of plastic samples recovered from *N. norvegicus* gut content analysis, discussed in Chapter Two, revealed a mix of mainly Nylon and polypropylene. Nylon is often used in maritime ropes that require a higher breaking strain and increased elasticity, such as cod-end rope, while polypropylene is commonly used in ropes and fishing gear due to it being less expensive than Nylon.

In addition to marine plastic litter resulting from accidental loss of sheets and rigging from vessels, fragments of polymer ropes can be released in to the environment through day to day fishing activities. Weathering of fishing gear

caused by typical use may contribute to microplastic releases. The action of trawling causes a high degree of abrasion to nets, to help protect their gear fishermen use sacrificial bundles of rope, known as chafers, to absorb the damage from the seabed. These ropes can contribute a high proportion of microplastics released into the marine environment.

Degradation and fragmentation also influences the fate of marine plastic debris. As discussed in previous chapters, the breakdown of plastic debris can influence the uptake of plastics, and the way by which microplastics affect the contact organism (Cole et al., 2011). For example, marine mammals are known to become entangled by macroplastics and to suffer damage when they are ingested (Gregory, 2009); however, the reduced size of microplastics allows them to be egested, reducing their impact on the organism (Eriksson and Burton, 2003). Unfortunately, as the potential for retention by large vertebrates is reduced, microplastics become available for ingestion by smaller organisms, such as fish and birds. Further fragmentation results in ingestion by planktonic copepods, tunicates, bivalves and crustaceans (Cole et al., 2013), and the potential for plastics to pass through membranes and into tissues (Browne et al., 2008; Farrell and Nelson, 2013).

As a result of their altered dimensions, fragmented plastics may exhibit a different distribution in the water column to that of macroplastic debris; this is caused by microplastics sinking at differing rates and being affected differently by the currents (Kukulka et al., 2012). This affects the range over which fragments accumulate in sediments (Kukulka et al., 2012; Kusui and Noda, 2003; Williams and Tudor, 2001). Once settled, these fragments affect a different range of organisms than macroplastic debris.

3.1.1 Degradation of Plastics in the Marine Environment

The effects of degradation factors on plastic materials can be observed in a number of ways, for example, visual degradation including; embrittlement, yellowing, and cracking (Massey, 2006); fragmentation (Barnes et al., 2009); and reduction in mechanical properties, for example ductility – the ease at which it is deformed under tensile stress (Massey, 2006). These impacts can be regularly observed on stranded plastics around the CSA (Figure 3.1).

Figure 3.1 Shoreline degraded plastic rope showing embrittlement and yellowing

Polymer degradation takes place in a three step process. The first stage, initiation, is characterised by the scission of the polymer chain either at the chain-end or randomly throughout its length, resulting in the formation of two free radicals. Free radicals are atoms, mollecules, or ions which containing unpaired electrons or an open electron shell; this makes the resulting molecules highly reactive. During the second reaction phase, propagation, radical groups act upon the hydrocarbon chain to cause further breakdown of the polymer

(Leonas and Gorden, 1993; Muasher and Sain, 2006). In the final stage, termination, radical groups may either recombine with the polymer chain or react to form new, stable, species (Faravelli et al., 2001). To date, a number of mechanisms responsible for the degradation of plastic have been described (Kinmonth, 1964), the most common are outlined below.

3.1.2 Light

Photodegradation is the action of light on a polymer (Andrady, 2011). The energy provided by UV and near UV light initiates the breakdown of the polymer backbone, either at the C-H, C=C and C-C bonds; or the sites of photosensitizers and catalysts (Singh and Sharma, 2008). The absorbed energy causes the formation of a short-lived singlet, which quickly forms a more stable excited triplet. In polymers containing aldehydes and ketones this can be observed in one of two Norrish reactions (Figure3.2), in which the triplet either cleaves the polymer chain in a Norrish type I reaction, or forms pairs of saturated and unsaturated chain ends – a Norrish type II reaction.

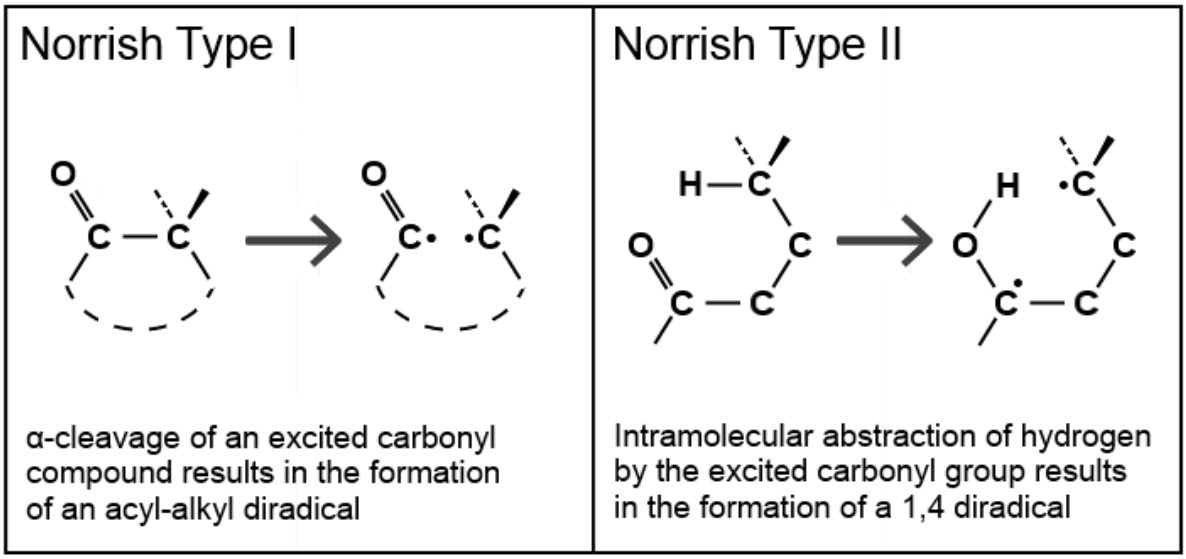


Figure 3.2 Norrish Reactions

During Norrish type 1 reactions acyl-alkyl radical pairs or acyl-alkyl di-radical pairs are formed via α-cleavage of an acyclic or cyclic carbonyl compound respectively. Under Norrish type 2 conditions an 1,4-diradical is produced via the abstraction of a *γ*-hydrogen by an excited carbonyl compound (IUPAC, 1997), whilst this does result in breaking the polymer backbone it does not produce the free radicals required for continued oxidation (Geuskens and David, 1979).

During the propagation phase, radical groups act upon the chain from which they were removed, or a neighbouring polymer chain; the overall number of free radicals remains constant. This process of auto-oxidation continues until the termination phase, when the free radical groups recombine, either as shortened polymers, stable species, for example a ketone and an alcohol (Geuskens and David, 1979), or in a mineralised state.

Samples of LDPE and LDPE with photosensitizers, exposed to UV irradiation over 10 years showed three stages of degradation. These were thought to represent a rapid change within the material and the development of equilibrium, followed by a reduced rate of degradation, and finally a collapse of the polymer structure (Albertsson and Karlsson, 1988). Because of the low penetration of light, most photochemical reactions take place on the surface of the plastic (Tyler, 2004); even so, photodegradation is considered to be the primary cause of plastic break down (Singh and Sharma, 2008).

3.1.3 Temperature

Thermal degradation is caused by high temperatures, and is almost exclusively the result of human action (Andrady, 2011). The activation energy required for the initiation phase is provided by heat, and – unlike photo-degradation, which only occurs on the surface – acts throughout the plastic (Singh and Sharma, 2008). For many of the most common polymers, thermal degradation begins at temperatures between 150 and 200°C, and increases in rate with temperature (Kholodovych and Welsh, 2007). Formation of radical groups occurs both by random scission throughout the polymer backbone, and chain-end scission of C-C bonds (Murata et al., 2002). This is followed by a propagation phase consisting of

a sequence of hydrogen abstractions, either between molecules or between groups within one molecule (Faravelli et al., 2001). As with photodegradation, termination occurs in a combination of radical groups (Singh and Sharma, 2008).

Much of the information available concerning thermal degradation is the result of studies into the effectiveness of pyrolysis and gasification in reclaiming energy and chemicals from plastics (Faravelli et al., 2001; Potts, 1970), and on the effects of fires and the production of toxic substances (Nyden and Noid, 1991). These provide insight into the chemical processes involved in thermal degradation, the expected products (Faravelli et al., 2003; Faravelli et al., 2001), and the activation energy of de-polymerisation reactions (Nam and Seferis, 1991), as well as identifying possible catalysts (Audisio et al., 1984).

Modelling has indicated that polymers of increasing molecular weight have decreasing thermal stability, as the number of bonds available for scission increases with the molecular weight; however, this has not been found to influence the rate of degradation (Nyden and Noid, 1991). Thermogravimetric analysis of high density polyethylene, low density polyethylene and linear low density polyethylene has demonstrated that branched polymers require higher activation energy. It was also observed that the extent of branching influences the reaction order, how the concentration of species interacts with reaction rate (Woo Park et al., 2000).

3.1.4 Oxygen and Ozone

Oxidative degradation is caused by the action of oxygen, ozone and other oxidizers on a polymer (Andrady, 2011). Although not yet fully understood (Fairgrieve, 2009), it is believed the initiation stage forms a radical peroxy (-OOH) group which attacks C-H bonds in a two stage process to form further peroxy radicals (Razumovskii et al., 1971). The reaction is regarded as autocatalytic, increasing in speed during the propagation phase as more peroxy radical groups are produced (Kholodovych and Welsh, 2007). Oxidation occurs first at labile, less stable, groups, resulting in certain polymers being more

susceptible to oxidation, for example polyethylene and polyether polyurethanes (SuPing and Darrel, 2009).

3.1.5 Water

The action of water on a polymer is known as hydrolysis (Andrady, 2011). As with oxidation, a polymer must contain labile functional groups, for example esters which form ionized acids. The rate of degradation is dependent on the hydrophobic nature of the polymer structure (Göpferich, 1996).

In some polymers, for example polyester, simple hydrolysis is the main degradation route (Figure 3.3). The first stage of the degradation process involves non-enzymatic, random hydrolytic ester cleavage and its duration is determined by the initial molecular weight of the polymer as well as its chemical structure (Pitt et al., 1981). Hydrolysis is especially important in the design of degradable polymers and many have increased numbers of hydrolysable bonds and a more hydrophilic structure, which increases the rate of polymer breakdown (Göpferich, 1996).

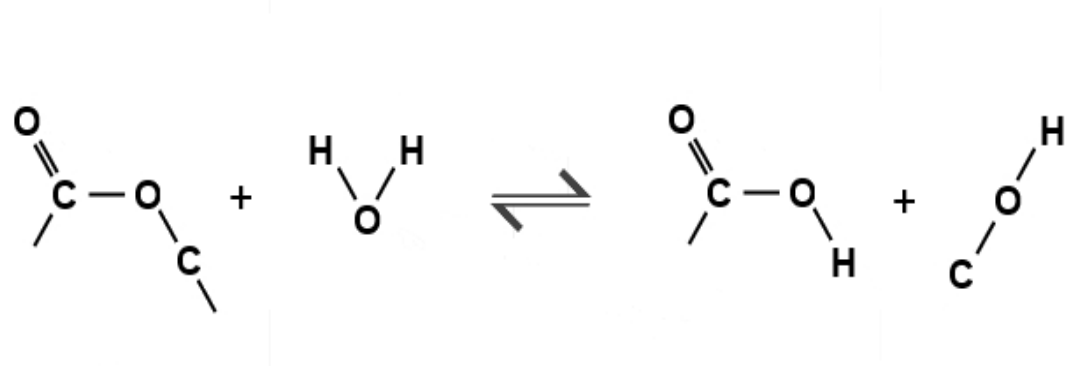


Figure 3.3 Hydrolysis of Esters in the Presence of Water

3.1.6 Biodegradation and Biofouling

Plastics in the marine environment are frequently subjected to colonisation by a range of organisms (Harms, 1990). Fouled plastic is susceptible to biodegradation, or biodeterioration, the effect of an organism and its by-products on a polymer (Andrady, 2011). As well as the mechanical action of borers (Davidson, 2012) a polymer may be subject to increased solubility, ionization (Singh and Sharma, 2008), and hydrolysis (Göpferich, 1996).

To enable the biodegradation of thermoplastics without weak functional groups, such as polyethylene, there must first be a reduction in the weight of the polymer by one of the methods described above (Bonhomme et al., 2003; Palmisano and Pettigrew, 1992). Enzymes produced by colonisers may then act as catalysts in a number of reactions, enabling the breakdown of polymers into their constituent oligomers (Flemming, 1998; Gu and Gu, 2005). This has previously been seen in samples of polyethylene colonised by the bacteria species, *Rhodococcus rhodochrous*, *Nocardia asteroids* and *Cladosporium cladosporoides*, which showed notable surface deterioration (Bonhomme et al., 2003). Biofouling may also weaken a polymer by acting upon additives and other associated compounds (Singh and Sharma, 2008). Microbes are known to attack fillers, such as starch, and other additives resulting in embrittlement and weakening of the plastic structure (Flemming, 1998; Morton and Surman, 1994).

The extent to which plastics may be affected by an organism is determined by a number of factors, the dimensions of the plastic item, the hydrophobic nature of a polymer (Hueck, 2001; Zheng et al., 2005), its molecular weight (Hueck, 2001; Potts, 1970), presence of additives of lower molecular weight, and the availability of substrate (Kawai, 1995). As such, certain polymers will be more susceptible to biodegradation, for example, the degradation rate of Nylon 66 has been observed to be affected by papain, tryspin and chymotrypsin; however, in the same experiment none of the enzymes chosen were found to affect poly(methyl methacryalate) (Smith et al., 1987). However, biofouling also has the potential to reduce the rate of photodeteriation, either by covering the surface of the plastic, or by reducing its buoyancy, thus reducing the level of UV light it is exposed to (Andrady, 2011; Ye and Andrady, 1991).

3.1.7 Variability

The number of degradation mechanisms and the range of recalcitrance exhibited by polymers results in high variability in the rates of plastic degradation in the marine environment (Albertsson and Karlsson, 1988). Heterogeneity in environmental factors may occur both seasonally, for example increased UV in summer (Massey, 2006) and variation in colonisation and growth rate of fouling organisms (Saldanha et al., 2003); and spatially, with different colonizers at different latitudes (Gregory, 2009).

Interaction between mechanisms may also influence the degradation rate. For example, the dependence of biodegradation on a prior decrease in a polymer's molecular weight (Palmisano and Pettigrew, 1992), usually by photodegradation (Albertsson and Karlsson, 1990); however, microbial growth has been observed on polyethylene that has not been pre-oxidized (Bonhomme et al., 2003). Biodeterioration may also be enhanced by the addition of water. Degradation rates of plastics colonised by fungi were found to increase with the addition of water (Albertsson and Karlsson, 1988).

3.1.8 Aims and Objectives

Whilst there is an increasing amount of information available on the levels of microplastic in the marine environment, there is little available data concerning the rate of microplastic input into the marine environment. In this chapter we aim to examine the weathering rates of commonly used polymers in the CSA in order to determine the potential microplastic inputs from commonly used polymers. The reduction in a number of mechanical properties - tensile strength, elongation at break, and reduction in sample weight – were used to determine the rate of degradation of plastics exposed to benthic conditions in the CSA. Changes in these three variables were compared to monthly variation in abiotic conditions and degree of biofouling to determine the factors responsible for reduction in mechanical properties of plastics.

3.2 Methods

3.2.1 Sample Preparation

In order to determine local breakdown rates of plastics, three polymers commonly identified in the gut contents of *N. norvegicus* examined in Chapter Two were exposed to benthic conditions in the CSA. Polyethylene, polypropylene, and Nylon ropes, purchased from Gaelforce, were selected to represent polymer ropes commonly used in recreational and industrial maritime activities. Natural sisal ropes were also used as a comparison to polymer materials.

Rope samples measured approximately 10 mm in diameter and were cut into 50 cm lengths, in order to ensure equal surface area. Each length was weighed, before being mounted on ABS frames in a randomised pattern (Figure 3.4). The frames were then placed on silty sediment at a depth of 10 metres over a period of 12 months, from the first of September 2012 to the thirtieth of August 2013.

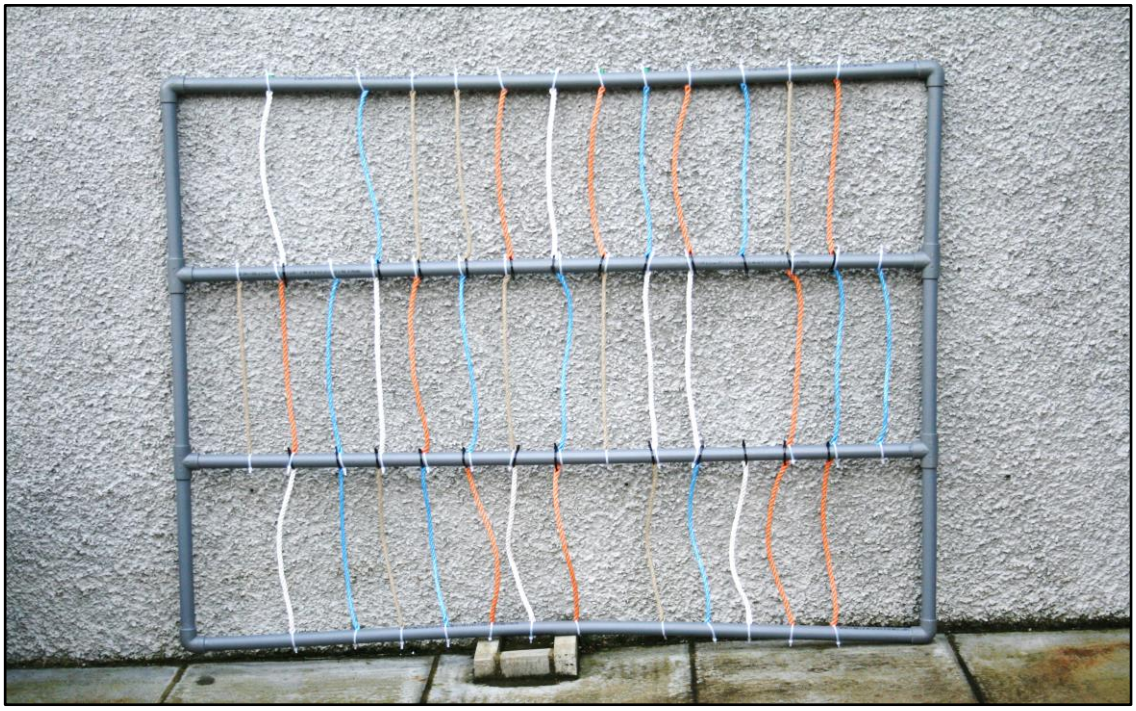


Figure 3.4 Ropes Mounted on ABS Frame Prior to Exposure

3.2.2 Monitoring Degradation Factors

Over the 12 month exposure period, daily temperature and light intensity – both previously seen to influence polymer degradation – were recorded using "HOBO temperature/light loggers" (model number: UA-002-64) attached to the suspended ABS frames. Every two months the frames were lifted to the surface and two lengths of each rope type were selected at random, removed from the frames, and analysed for the level of colonisation and weathering.

The degree of biofouling was examined by measuring primary production and the diversity of the fouling organisms. Primary production was measured using level of chlorophyll *a*; 10 mm lengths were placed in individual containers with 10ml of 90% acetone and refrigerated. After 24 hours, each sample was centrifuged for 10 minutes, and the resulting supernatant was transferred to a 1 ml cuvette. The chlorophyll concentration was determined by using a Thermospectronic HeliosΥ spectrophotometer, with readings taken at 750, 664, 647, and 630 µm, using a 90% acetone blank.

The abundance and type of fouling organisms was determined in by enumerating the number of colonising organisms and the weight of attached biomass. Biofouling by macro-organisms was determined by sampling 50mm sections of rope. These were examined under a binocular microscope and any algae and animals were removed for identification. Attached macro-algae were removed and oven dried at 40ºC overnight to determine the dry weight on each polymer type.

3.2.3 Measuring Sample Degradation

Rope samples were also examined for evidence of weathering. The change in sample weight was used to determine the mass of the sample lost as a result of fragmentation. Other properties previously shown to vary with increased exposure were buoyancy and tensile properties. Changes in the buoyancy of the polymer were calculated by first determining the volume of the sample by total submersion in water, then floating the sample in a saturated saline solution. The

volume of the sample was multiplied by the density of the saline solution to give the mass of fluid displaced.

Changes in the mechanical properties of the polymer were examined by tensile testing – elongating a specimen and testing its load bearing capacity (McKeen, 2008). Baseline tensile strength was determined by testing un-weathered lengths of each rope type. Bimonthly samples were rinsed with deionised water and air dried, before storing in the dark at 20ºC until the time of analysis. Testing was carried out using an Zwick-Roell Z250 tensile testing machine with a capacity of 100kN (Breslin and Li, 1993; McKeen, 2008). Samples were mounted between the two flat crossheads, and subjected to tension. Tensile strength was calculated by determining the force per unit area required to fracture the sample. Determination of upper and lower yield strength is taken from plots of engineering stress and engineering strain. Elongation is recorded as both the total increase in length and the ratio between the change in length and the original length, "strain".

3.2.4 Abiotic Conditions during the Exposure Period

Between September 2012 and August 2013 the average sea temperature was 9.73 ºC. The minimum temperature was 5.66 ºC, recorded in March; this coincided with a period of cold weather. The maximum temperature was 17.9 ºC recorded in July. Average light intensity over the exposure period was 122 Lux, reaching a maximum of 17222 Lux, in June, and the highest monthly average light intensity took place throughout June and July.

3.2.5 Statistical Analysis

The statistical significance of the observed changes in the mechanical properties of each rope type was analysed using Minitab 15. Monthly variation in tensile strength, elongation at break, and sample weight were separately examined using Kruskall-Wallis analysis.

The effect of environmental factors on both tensile strength and elongation at break were examined using GLM in R (version 3.0.2). A number of variables, for example maximum and average light intensity, were found to inter-correlate; these variables were included in sequential models to determine which had the greatest impact on model fit. After running a GLM using all environmental variables, the model was reduced using in a stepwise process in order to improve the AIC of the resulting model. Analysis of variance was then used to identify the variables which had a significant effect on the rope samples.

3.3 Results

3.3.1 Variation in Mechanical Properties

The change in tensile properties observed in the rope samples was highly variable; however, all materials demonstrated a reduction in sample weight during the sample period. Of the three polymer ropes, polyethylene showed the highest average change in elongation at break, followed by polypropylene and Nylon (Figure 3.5). Mean change in tensile strength was highest in polypropylene, followed by Nylon and polyethylene (Figure 3.6). For all rope types, the rate of degradation was greatest in the first months, slowing during the 12 month experimental period.

The weight and percentage mass lost by each rope type was also highly variable (Figure 3.7). Average monthly reduction in the weight of Nylon samples was 0.422g (1.02% of sample by weight). When Nylon line, a fibrous rope, was examined under SEM, there was obvious increased fraying; fibres did not lay as flat to the rope surface, and there were notable breakages of individual strands (Figure 3.8). Extruded polyethylene filament rope lost an average of 0.132g (0.45% of sample) per month. Visual analysis of the rope's condition revealed increased surface scratching and roughening over the 12 month period (Figure 3.8). Polypropylene lost an average of 0.086g per month (0.39% of the sample weight). After 12 months, the surface of the twisted film rope developed many

visible cracks and fissures, as well as the formation of fine surface fibres - particularly apparent in areas of animal attachment (Figure 3.8).

The natural rope, sisal, lost 100 percent of its mass between six and eight months, as a result its average mass lost was over 12.5% per month. Analysis of the reduction in mechanical properties revealed significant reductions in both elongation and breaking strain.

3.3.2 Biofouling Organisms

Sisal was by far the most degradable rope, with no trace recovered when the frames were lifted in month eight. Examination of the rope samples for the presence of fouling organisms showed very little colonisation. Samples recovered two, four, and six months into the sample period had low chlorophyll *a* readings and less than 0.01g dry weight of macroalgae – which was first observed after four months. Only two faunal groups were found to have colonised sisal; amphipods, *Corophium*, present after two months, and Chironomid fly larvae two individuals, present after four months.

All polymer rope samples exhibited some degree of biofouling, however, the number and type of fouling organisms was variable. The amount of macroalgae recovered greatly increased over the course of the year, although the rate of growth was lower on Nylon samples. Macroalgae first appeared on polypropylene samples after four months of exposure. Growth on polypropylene samples reached 10.7g dry weight per 50 mm of rope, the highest recorded on all polymers. Macroalgal growth on polyethylene samples was first recorded at six months, and resulted in 9.3 g dry weight, and on Nylon samples was first recorded at eight months and reached a weight of only 2.05g dry weight. The two most commonly identified macroalgal species were *Alaria esculenta* and *Palmaria palmata*. *Palmaria* was observed on all rope types, occurring after eight months on polymer ropes. *Alaria* was only observed on polypropylene and polyethylene ropes, first recorded on polyethylene at six months, followed by polypropylene at eight months. The high algal dry weights observed on these two polymers were the result of large *Alaria* fronds.

The number of observed invertebrate species varied between rope types. Sisal demonstrated the lowest number of colonisers, with only *Corophium* at two and four months, and *Corophium* and *Chironomid* at six months. It is currently unclear as to whether this lower figure is the result of the unstable rope surface, with strands collapsing before attachment, or the result of fewer planktonic larvae early in the exposure period.

The rate of colonization varied between the three polymer ropes. All polymer ropes were colonised by *Corophium* at two months. On polyethylene samples, four species were observed at six and eight months, subsequently falling to three species. The maximum number of species observed on Nylon was also four, observed at ten months. Polypropylene had the highest number of colonizers, five species, observed at ten and twelve months.

The number and type of invertebrate organisms observed varied over the course of the study period. The most common colonisers of all rope types were *Corophium*, which formed sand tubes on the rope surface. *Corophium* were observed in highest numbers on sisal after four months. This may be due to the increased roughness and high level of biofouling of the sisal rope. At between six and eight months the number of grazers was observed to increase with the appearance of the periwinkle, *Littorina littorea*. A late coloniser of polyethylene was *Stenula*, an amphipod commonly found in sublittoral alage, possible attracted by increasing macroalgal cover.

Prolonged exposure to benthic conditions resulted in fouling by larger encrusting organisms. The barnacle, *Eliminus modestus*, was found on samples of polypropylene exposed for over eight months, and the blue mussel, *Mytilus edulis*, was found on all polymers between six and eight months exposure. After 12 months polypropylene had the most recorded species with five per 50mm rope sample, while Nylon had only four species recorded per 50mm sample. However, the most colonised plastic was observed to be the data loggers, which exhibited a much more complex community than that of the rope samples.

3.3.3 Analysis of Factors Influencing Reduction in Mechanical Properties

Prior to statistical analysis, the independent variables were analysed for inter-correlation. This revealed significantly similar distributions between exposure period and maximum light intensity, minimum and average temperature, and maximum and average light intensity. As a result, average light intensity and average temperature were used for statistical analysis. The reduction in the tensile strength and elongation at break of each polymer was compared to abiotic conditions and colonisation by biota in a pair of GLMs. The results of which indicated that the potential causes of degradation varied between polymers.

The GLM of both elongation and tensile strength of all three polymer ropes exhibited different responses, however, a number of factors were common between the three materials (Tables 3.1 – 3.5). There was no relationship observed between any of the monitored environmental factors and the tensile strength of Nylon rope samples. Reduction in the mechanical properties of sisal rope was linked to the exposure period and the average temperature. Extruded polyethylene and Nylon fibre ropes were seen to be susceptible to increasing temperature, whilst reduction in the mechanical properties of twisted polypropylene film rope was found to be linked to changes in the average light intensity.

The impact of associated biota was also seen to vary between rope types. Whilst all were seen to significantly affect elongation at break, this was not the case for tensile strength. The weight of attached algae was seen to significantly influence the level of elongation at break in both polypropylene and polyethylene samples.

Table 3.1 Factors Correlated with Changes in Elongation at Break of Polypropylene

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Exposure	1	1621.1	19	23095.6	**0.0004**
Average Temp	1	4713.3	18	18382.2	**2.20E-09**
Max Temp	1	13041.2	17	5341.1	**2.20E-16**
Macroalgae	1	3230	16	2110.4	**7.40E-07**

Table 3.2 Factors Correlated with Changes in Elongation at Break of Polyethylene

	Df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Species	1	41.2	19	3999.4	0.531515
Average Temp	1	447.19	18	3552.3	**3.91E-02**
Max Temp	1	254.56	17	3297.7	1.20E-01
Average Light	1	496.55	16	2801.1	**2.97E-02**
Macroalgae	1	1225.82	15	1575.3	**6.35E-04**

Table 3.3 Factors Correlated with Changes in Elongation at Break of Nylon

	Df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Species	1	2375.9	19	5478.6	**0.001745**
Max Temp	1	1115	18	4363.7	**0.031988**

Table 3.4 Factors Correlated with Changes in the Tensile Strength of Polypropylene

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Species	1	160330	19	1764580	**0.095196**
Max Temp	1	257605	18	1506975	**3.44E-02**
Average Light	1	528027	17	978948	**2.46E-03**

Table 3.5 Factors Correlated with Changes in the Tensile Strength of Polyethylene

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Species	1	134327	19	1476314	0.15731
Average Temp	1	28792	18	1447522	5.13E-01
Max Temp	1	144785	17	1302737	1.42E-01
Average Light	1	185061	16	1117676	**0.09694**
Macroalgae	1	110153	15	1007523	0.20033

3.3.4 Analysis of Factors Influencing Fragmentation

Kruskall-Wallis analysis of the percentage mass lost by each polymer per month indicated significant differences in the fragmentation of each rope type ($H = 18.23$, $df = 3$, $P < 0.001$). GLM analysis was then used to determine the factors responsible for the degradation of individual polymers. The average mass lost per month was compared to changes in the tensile properties of the sample and the colonising organisms.

The fragmentation of polypropylene rope samples was not significantly related to any of the measured variables, although a number of weak relationships were apparent (Table 3.6). The fragmentation of polyethylene samples was seen to be linked to a reduction in elongation at break and the level of chlorophyll a recorded (Table 3.7). Statistically significant relationships were observed between the monthly rate of fragmentation of Nylon samples and reduction in elongation at break and the weight of attached macroalgae (Table 3.8).

Table 3.6 Factors Correlated with Changes in the Weight of Polypropylene

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Tensile Strength	1	1.50758	16	22.617	0.3546
Elongation	1	0.096	15	22.521	8.15E-01
Chlorophyl a	1	0.17209	14	22.349	7.55E-01
Macroalgae	1	0.10395	13	22.245	8.08E-01
Macroinvertebrates	1	2.17181	12	20.073	0.2666
Exposure	1	0.71944	11	19.354	0.5225

Table 3.7 Factors Correlated with Changes in the Weight of Polyethylene

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Tensile Strength	1	0.05955	16	11.2093	0.73148
Elongation	1	1.69446	15	9.5148	**6.72E-02**
Chlorophyl a	1	0.89033	14	8.6245	1.85E-01
Macroalgae	1	1.32309	13	7.3014	1.06E-01
Macroinvertebrates	1	1.61276	12	5.6886	**0.07411**
Exposure	1	0.12653	11	5.5621	0.61691

Table 3.8 Factors Correlated with Changes in the Weight of Nylon

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Tensile Strength	1	1.8272	16	15.5263	0.10567
Elongation	1	4.5049	15	11.0214	**0.01107**
Chlorophyl a	1	0.12	14	10.9014	0.67841
Macroalgae	1	2.825	13	8.0764	**0.04424**
Macroinvertebrates	1	0.2621	12	7.8144	0.54005
Exposure	1	0.1364	11	7.678	**0.65845**

3.4 Discussion

3.4.1 Change in the Mechanical Properties of Ropes Weathered in the CSA

Over the 12 month experimental period all rope types exhibited a reduction in sample weight. This was most noticeable in sisal, the samples of which had all completely degraded after six months, with no trace recovered when the frames were lifted subsequently. As expected, polymer ropes proved to be far more durable, with all polymers exhibiting minimal reductions in sample weight. Nylon showed the greatest weight loss of the polymer ropes, averaging 1.02% per

month. The next greatest weight reduction was observed in polyethylene, with an average monthly loss of 0.45%, then polypropylene with 0.39%.

3.4.2 Factors Affecting Reduction in Mechanical Properties

Unsurprisingly, given its rapid degradation, the change in mechanical properties was greatest in sisal samples, which exhibited significant reductions in both breaking strain and elongation at break. The resulting GLM analysis revealed that exposure period was the most important factor in the degradation of sisal rope.

The change in tensile properties differed between polymers. There were significant reductions in elongation at break in both polypropylene and polyethylene, indicating that both polymer types demonstrated reduced elasticity, but no significant change in tensile strength. Increased tensile strength has been observed in earlier studies, and is believed to be the result of increased cross linkages between polymer chains as free radical groups join to form stable molecules (Whitney et al., 1993).

The GLM analyses of the factors responsible for change in the mechanical properties of both polypropylene and polyethylene rope samples returned similar results. Light intensity was identified as a factor in the reduction in strength of both polypropylene and polyethylene rope. As discussed in the introduction, the action of light results in the degradation of polymers by exciting bonds in the polymer chain causing them to split. The free radicals created in this reaction go on to affect other bonds in the chain, until they form new, stable products (Andrady, 2011; Singh and Sharma, 2008).

Of the polymers tested, polypropylene and polyethylene have a poorer UV resistance than that of Nylon. This may explain the lack of UV response observed in Nylon, especially when considering the buffering effect of seawater. Due to the surface refraction and absorbance by seawater, the impact of UV light would diminish with increasing depth. This would reduce the rate of UV damage to the structure of all polymers in deeper water; halting it entirely below the photic zone.

Reduction in the elongation of all polymers was seen to be related to temperature. As discussed in the introduction, temperature is known to influence the rate of polymer breakdown. Firstly, by exciting electrons within the polymer structure, this results in bond scission and the shortening of the polymer chain (Singh and Sharma, 2008). Secondly, increased temperature affects the rate of other forms of degradation; for example, if background energy is higher, this will reduce the light energy required to cause scission of vulnerable bonds (Singh and Sharma, 2008). In examination of the degradation rates of polyethylene films, temperature was seen to significantly influence the reduction in tensile properties (Whitney et al., 1993).

The effect of temperature will be greatly dependent on local climate and bathymetry. Smaller water bodies will be more greatly affected by seasonal fluctuations in air temperature, whilst deeper regions have more stable temperature regimes. In the Clyde, increasing depth would be expected to result in a stabilisation in the effect of temperature on degradation, as the overlying water provides a buffer to seasonal changes in air temperature.

The GLM analysis of the reduction in mechanical properties of Nylon samples also highlighted a relationship with the number of associated invertebrate species. The type and number of invertebrates was similar on all polymer ropes, suggesting that the structure of Nylon may be more susceptible to breakdown by fouling organisms.

The impact of invertebrate colonisers and other fouling organisms on mechanical properties is believed to be the result of constitutive enzymes on the surface of the polymer. These chemicals act on the bonds in the polymer to weaken them (Flemming, 1998; Göpferich, 1996; Gu and Gu, 2005). Break down of these bonds results in shortening of the polymer backbone which enables biodegradation by other organisms (Bonhomme et al., 2003), as well as an increase in the surface solubility of the polymer, enabling other organisms to attach themselves to the rope. The comparatively small reduction in the mechanical properties observed in Nylon rope samples would be expected from biochemical weakening of the surface layer rather than even degradation throughout the rope. The rate of

degradation by surface colonisers is limited by the surface area available and the number of potential colonisers in the water column.

3.4.3 Factors Affecting Reduction in Sample Weight

The average percentage mass lost over the exposure period varied between rope types, with natural sisal showing significantly higher rates of fragmentation than those of polymer ropes. The rate of degradation of sisal increased over the eight month monitoring period, however, the rate of degradation observed in polymers varied between samples.

It may be expected that a greater reduction in mechanical properties would correspond to greater rates of fragmentation; however this was not the case. Despite having the smallest reduction in mechanical properties, Nylon fibre rope showed the highest level of fragmentation over the 12 month exposure period; this was followed by polyethylene, and then twisted polypropylene rope. GLM analysis revealed that the fragmentation of Nylon samples was seen to be statistically related to the reduction in sample elongation at break and the weight of macroalgae. It may be the case that further degradation of the polymer structure is required to sufficiently weaken the polymer chains to enable visible fragmentation as a result of biodegradation (Whitney et al., 1993).

The small reduction in the tensile properties of the polymer ropes suggests that the majority of mass lost by the samples is the result of mechanical abrasion. This may be the result of either contact with benthic or suspended sediment, or the action of biota. Stranded macroplastic debris from Hawaii has shown damage from large fish which have mistaken the objects for prey (Carson, 2013); a similar effect is likely in this case, feeding grazers incidentally abrade the rope surface, increasing the rate of fragmentation. The significance of macroalgae in the model results is believed to be the result of holdfasts damaging the fine fibres of the Nylon rope, either through their formation, or the dragging action of macroalgae against the friction forces of the currents.

Despite showing the greatest reduction in mechanical properties, polyethylene samples lost the least mass over the experimental period. Statistical analysis of the factors responsible for polyethylene sample fragmentation showed significant relationships between the weight of plastic lost per month and the reduction in elongation and level of chlorophyll *a*. The level of fragmentation observed in polyethylene may also be the result of the rope construction; the twisted rope having individual fibres which are more easily broken than the broad twisted film construction of polypropylene. These fibre ropes also present a comparatively larger surface area, resulting in a greater area exposed to abrasion.

Analysis of the measured factors responsible for the fragmentation of polypropylene revealed no statistically significant links. This suggests that fragmentation of polypropylene may be either solely caused by sediment and wave action on the rope, or that the effect of these mechanical weathering factors is masking any other relationship with the measured variables.

Fragmentation of the ropes' surface was observed in SEM images, which revealed changes in the appearance of the surface of all rope types. The nature of this surface damage varied between rope constructions. Over the 12 month period there was a notable roughening of the polymer surface of both extruded polyethylene and polypropylene film ropes (Figure 3.8). On polyethylene rope there appeared to wear though the individual fibres, on polypropylene film this roughening caused the formation of thin strands. Nylon rope, comprised of thin twisted strands showed increased numbers of loose fibres. The distinct differences in surface weathering between the different rope constructions indicates that some manufacturing methods result in more durable materials, potentially delaying microplastic formation. Whilst not significant in the case of lost rope, this may have implications for the formation of microplastics from ropes in use in the marine environment, such as creel ropes.

3.4.4 Formation and Fate of Microplastics in the CSA

Unlike in studies of degradation rates of films, in which fragmentation was comparatively rapid, the surface area to volume ratio of rope samples is small (Whitney et al., 1993). As a result, much of the mass of the sample would be protected from environmental conditions. The average rate of degradation observed over the 12 month experiment was between 0.42 and 0.08 g m^{-1} per month; however, as the rope begins to break up and the surface area to volume ratio increases the rate of degradation would be expected to increase accordingly (Andrady, 2011).

The results presented above indicate that changes in light levels and temperature have the biggest influence on the degradation of polymer rope; however, these results are only of academic value unless they can be applied over a broad geographic area. Average water temperature at the experimental site was found to be 9.81ºC, and ranged from 5.6 to 17.5 ºC; this range is similar to that previously reported by Slesser and Turrell (2005), which ranged from 6 - 15 ºC. Therefore, it is expected that the rates of temperature degradation observed here are normal for the CSA. With increasing depth these temperatures would be less affected by seasonal variation in air temperature as the upper layer of water forms a buffer, stabilizing the rate of thermal degradation throughout the year.

Similar variation can be observed in light intensity. Whilst the level of light reaching the sea bed varied greatly over the course of the year, the average was 122.8 lux. Although previously seen to have a significant impact on degradation rates in shallow sub-tidal areas, the average light level will decrease rapidly with depth - as seawater absorbs a greater proportion of light. As a result, the rate of UV degradation is expected to decrease with depth.

The results indicate that mechanical abrasion by sediment and the action of grazers is important in the early stages of rope fragmentation. One of the most common invertebrate colonisers of all rope types were *Corophium*. These grazing invertebrates feed on algae and, in the process, may take in microplastic fibres. This may both contribute to plastic fragmentation and be a primary route

by which plastics enter the food chain. *Corophium* is prey for a range of species including wading shorebirds (MacDonald et al., 2014; Wilson and Parker, 1996), the brown shrimp, *Crangon crangon*, shore crab, *Carcinus maenas,* (Emmerson and Raffaelli, 2004); this importance of *Corophium* in marine food webs indicates that this may a significant route for microplastic bioaccumulation.

In addition to *Corophium*, the range of fauna observed on all rope types are commonly found around the CSA. However, they exist within a defined area of intertidal and shallow subtidal waters. As a result, the impact of fouling organisms may be highly variable with increasing depth. A similar response will be observed in algal colonisation, as lower light will reduce the level of photosynthesis.

The occurrence of sessile organisms seen here was concurrent with that of settlement of planktonic larvae seen throughout the CSA. The timing of the appearance of these species may vary slightly between years, this may result in a minor change in the primary rate of degradation; however, over extended periods this effect should be negligible.

Over the 12 month exposure period average monthly air temperature ranged from 4.9 – 11.4 ºC, with 1271.3 sunshine hours recorded. These were consistent with local monthly averages recorded between 1981 and 2010: between 2.6 – 19.6 ºC, and 1320.0 hours of sunshine. This suggests that levels of photo degradation and temperature degradation in the CSA will be similar between years.

3.4.7 Comparison with other areas

Local atmospheric conditions are similar to the UK average for the same period (an average of between 0.9 – 19.6 ºC, and 1372.8 sunshine hours); however, small scale local factors such as variation in turbidity, the occurrence of thermal effluent or upwelling, and variation in substrate, will all influence the local rate of degradation. In examining the impact of degradation factors on

polyethylene films, minor differences in environmental conditions were seen to have significant effects on degradation rates (Whitney et al., 1993).

Outside the UK, comparison becomes yet more difficult. There are currently few studies on degradation rates of polymer ropes in marine conditions, and even fewer in benthic environments; however, as briefly covered in the section 3.1, rates of plastic degradation are known to vary with location. This variation is the result of a difference in local biotic and abiotic factors. Sites close to the equator are subject to higher levels of UV radiation, as less light is reflected by the ozone layer. As a result, the local rate of UV degradation is higher than that in higher latitudes (Kinmonth, 1964). Closer to the pole, not only is the transmittance of UV lower, but there is an associated decrease in temperature. As a result there is a much reduced level of thermodegradation. There are, of course, areas which do not fit within these trends. Areas of cold water upwelling, and those subject to warm currents, such as the North Atlantic current, will vary compared to adjacent areas. This variation in abiotic conditions also results in differing availability of organisms responsible for biofouling and polymer degradation. DNA identification of bacterial colonisers on marine debris has indicated spatial and temporal variation in community structure (Oberbeckmann et al., 2014).

Areas of high anthropogenic input of both primary and secondary microplastic pollution are known to display high levels of microplastic debris; however, areas of high macroplastic aggregation pose a potentially greater risk. The conditions which bring macroplastic to aggregation sites would prevent formed microplastic from leaving. As a result the volume of microplastic will increase annually. Should marine macroplastic debris aggregate in areas of high temperature and UV, the formation of microplastics will be increased.

The changing climate may result in changes to microplastic formation in different areas. Changes in abiotic conditions include variation in local temperatures and weather patterns (which will affect UV availability). As a result, there will be alterations in the range of fouling organisms. At this time, there is insufficient data to identify those areas which will become at greater

risk of secondary microplastic formation, and this is worth monitoring in the long term.

3.5 Summary

The rates of plastic degradation seen here are specific to the CSA, and are the result of a complex interaction between abiotic and biotic factors. The degradation rates of ropes presented here are much slower than that observed for films and pellets. We believe this to be the result of the ratio of exposed surface area to overall volume. It is believed that as ropes begin to degrade, increasing the SA:V ratio, the rate of degradation will increase accordingly.

The factor most closely correlated with degradation varied between polymer types. This may be a result of the different functional groups within the polymer. The difference in polymer structure results in different labile functional groups.

Little is known about the fate of microplastic fibres following formation. One of the most common invertebrate colonisers of all rope types, *Corophium* are known to graze on algae and in the process may take in microplastics from the polymer surface. *Corophium* are an important food source for many species, and may be a primary route by which plastics enter the food chain.

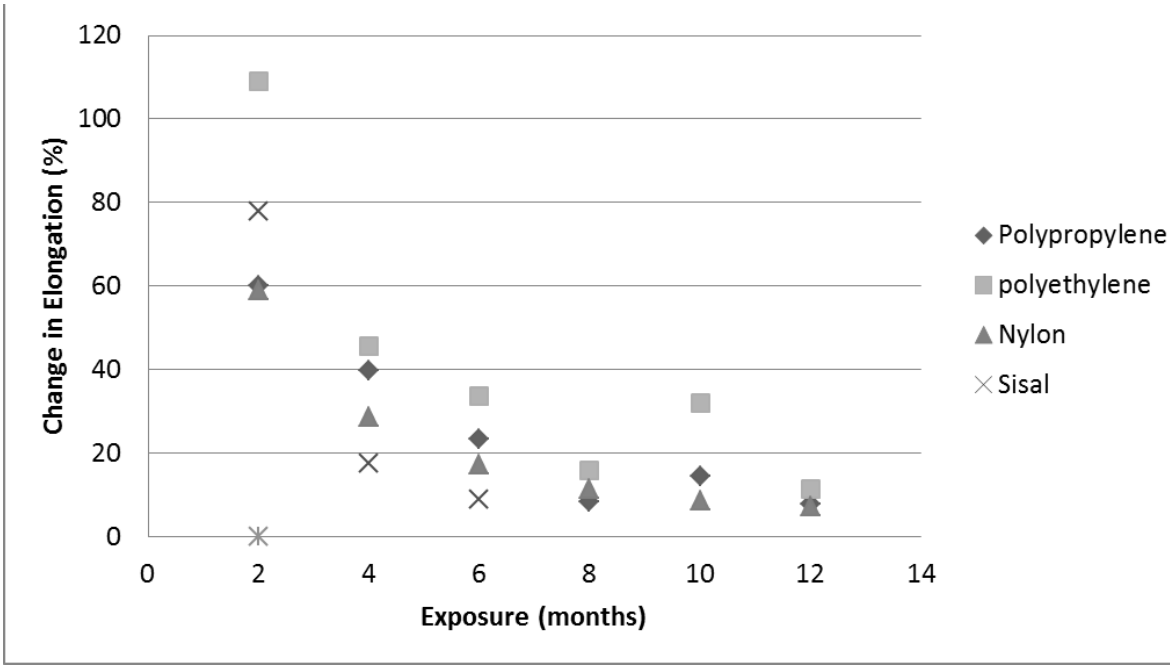


Figure 3.5 Change in the Elongation at Break of Each Polymer Type with Increasing Exposure

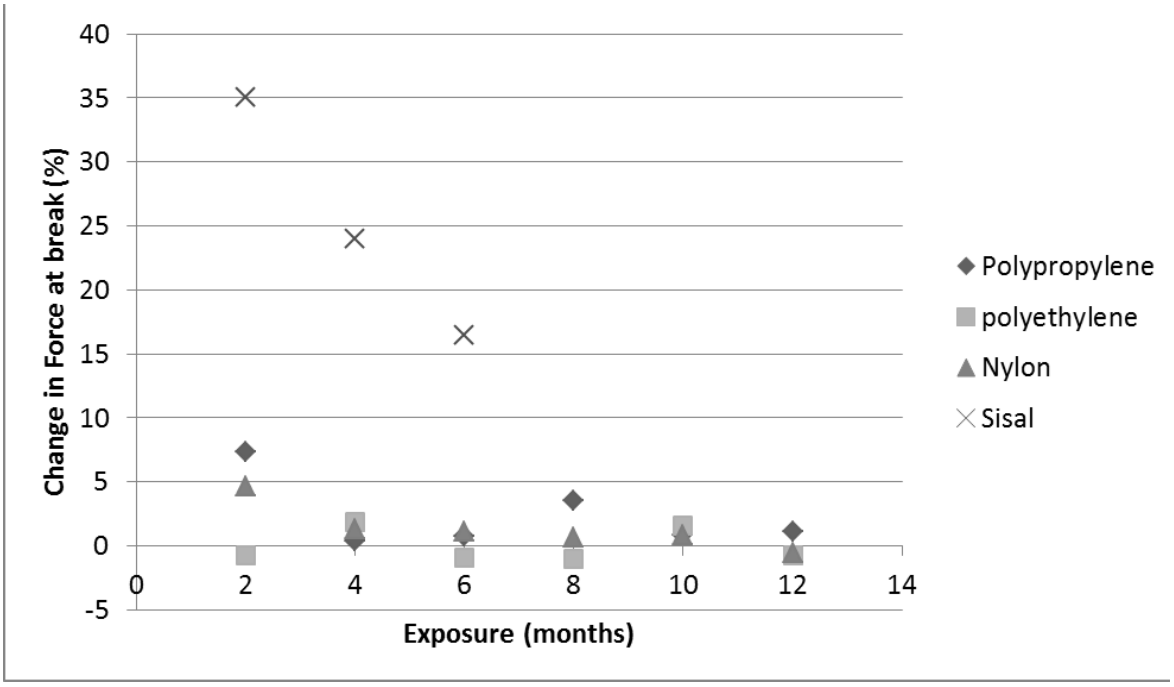


Figure 3.6 Change in the Tensile Strength of Each Polymer Type with Increasing Exposure

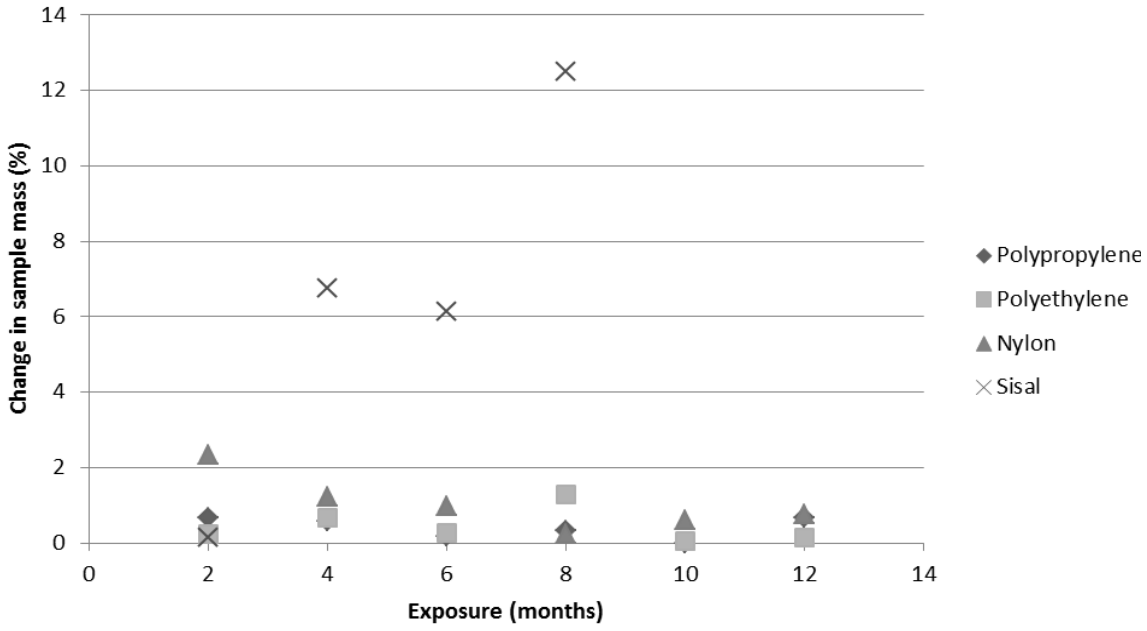
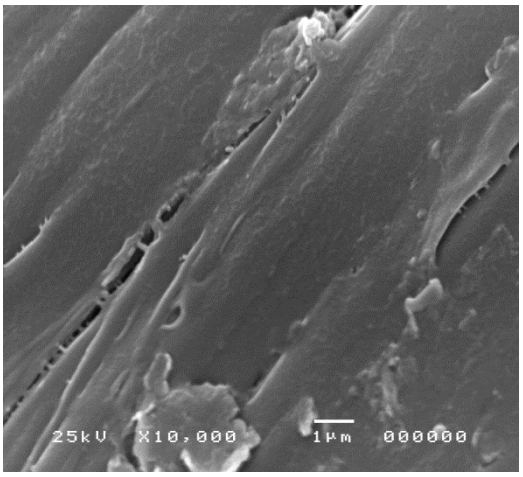
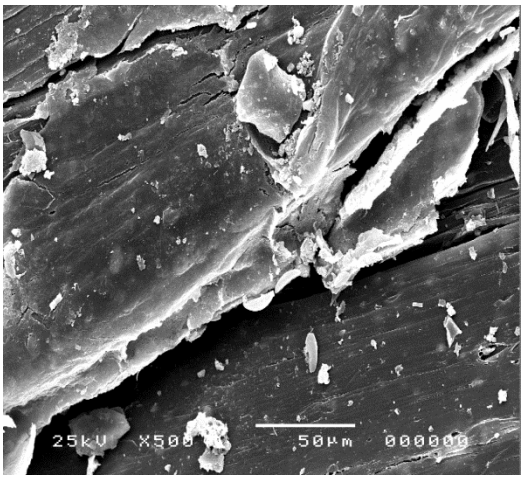
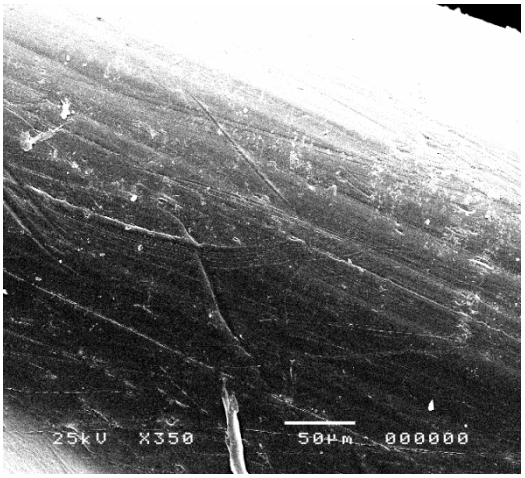


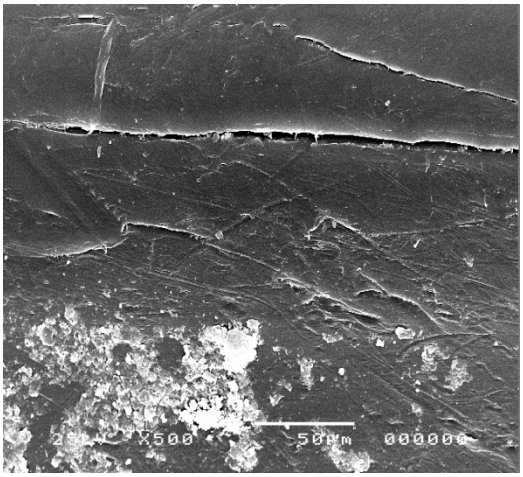
Figure 3.7 Percentage of Sample Mass Lost by Each Polymer Type with Increasing Exposure Time



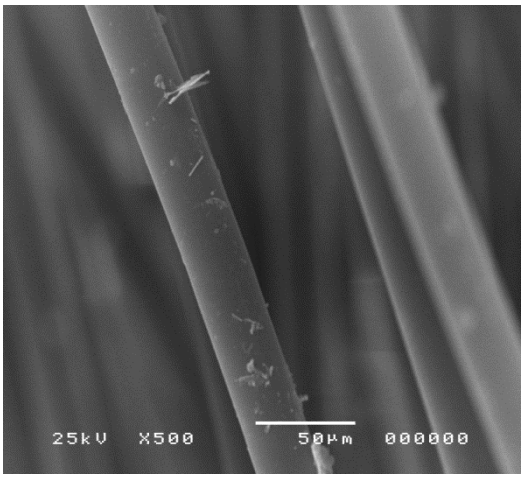


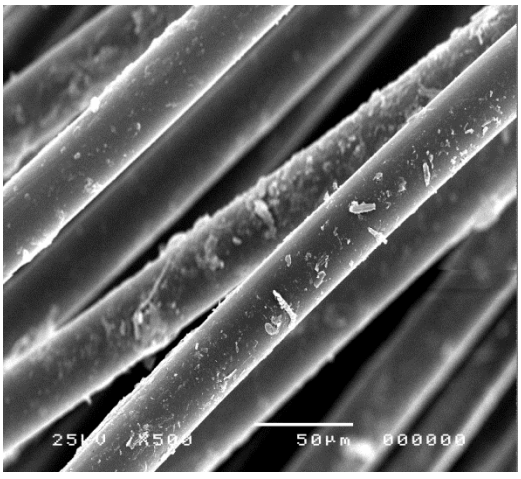
Polypropylene Before After





Polyethylene Before After





Nylon Before After

Figure 3.8 Changes in Polymer Surface of Rope Before and After 12 Months Exposure to Benthic Conditions

Chapter 4 Microplastic Distribution in the Clyde Sea Area

4.1 Introduction

In 2009, the minimum estimate of discarded plastic in Scotland was 336,622 tonnes. In the Clyde catchment, the high population density results in large amounts of plastic waste, around 49,887 tonnes from Glasgow alone (Weir et al., 2012). The catchment is subject to numerous diffuse and point sources of plastic pollution, the most common of which are wind-blown litter, sewage outfalls, and landfill runoff. This results in high riverine plastic loads, which travel downstream to contaminate coastal areas (Santos et al., 2009).

In addition to general household plastics from urbanised areas, certain areas worldwide are subject to equally detrimental site specific pollution (Yoon et al., 2010), and local industry (Kusui and Noda, 2003). In the CSA, site specific sources of pollution include fish farms, marinas, and many beaches popular with tourists which add to the litter released from common sources of plastic (Weir et al., 2012). In addition to local plastic inputs, diffuse plastic sources such as local fishing intensity and proximity to marinas and pleasure beaches can also have an effect on the composition of local marine litter in the CSA.

4.1.1 Movement of Marine Plastic Debris

After release into the environment plastics are able to disperse over long distances. Plastics have been shown to cross oceans (Martinez et al., 2009), travelling the circumference of basins on gyres (Kubota et al., 2005). In extreme cases, debris can travel outside the orbit of these gyres, moving to adjacent systems (Ebbesmeyer et al., 2007).

Whilst large scale mapping shows definite debris drift patterns, on a smaller scale, high variation has been observed in the distribution of both suspended (Dixon and Dixon, 1983), and deposited plastic (Claessens et al., 2011). This

variation is due to a complex relationship between anthropogenic inputs and environmental factors.

4.1.2 Horizontal Distribution

A number of variables affect the spatial range over which plastics are deposited. The most commonly identified factors in the horizontal movement of plastic debris are patterns of circulation and the effects of windage. These are most clearly visible in offshore movement of debris. Geostrophic currents (water flowing between pressure gradients) and the effect of wind, comprised of wind action on the exposed "float", Stokes drift (wind driven waves) and Ekman currents have all been seen to affect the movement of floating debris (Jonasson et al., 2007; Maximenko et al., 2011).

Global circulation patterns, the result of wind, temperature and salinity (Jonasson et al., 2007), have been seen to correlate with the movement of plastic children's toys lost from cargo ships in the North Atlantic (Ebbesmeyer et al., 2007). The impact of gyres in aggregating plastic is well known (Moore, 2008); the circulating currents cause large debris fields. Whilst gyres trap a large proportion of marine plastic, modelling indicates that the entrainment of a piece of plastic is dependent on its position within the vortex (Budnikov et al., 2012). Plastics have also been seen to accumulate at convergences (Law et al., 2010).

As well as the effects of wind driven currents, plastics riding on or slightly above the water's surface are subject to the friction effect of wind on the object's surface, known as windage (Shaw and Mapes, 1979). The course of an object subject to windage may be very different to one subject to the prevailing current alone. Rates of litter input on Tresilian Bay in South Wales have been correlated with wind speed (Williams and Tudor, 2001). A study comparing windward and leeward beaches in Curaçao showed that windward beaches exhibited 24.2% more plastic by abundance (Debrot et al., 1999). This distribution can also be observed in fragmented plastics; in the Tamar estuary, larger amounts of plastic were recovered at downwind sampling locations (Browne et al., 2011).

The degree to which debris is affected by the currents or windage is the result of a number of factors. The average buoyancy and shape of debris indicates how much will be above water for wind to affect it (Yoon et al., 2010). For example, the distribution of particles less than 1mm in size was clearest in denser plastics (Browne et al., 2011).

4.1.3 Vertical Distribution

Plastic load also varies vertically in the water column; dispersal of plastic particles is affected by the polymer's buoyancy and water turbulence (Lattin et al., 2004). When undisturbed, the salinity of seawater and a polymer's density dictate a fragment's position in the water column. High density results in negative buoyancy, which causes plastic to collect on the ocean floor (Galgani et al., 1996).

Buoyancy dependent distribution is disturbed by vertical mixing. The use of one dimensional column models has indicated that plastic debris is distributed vertically in the upper water column by wind driven mixing (Kukulka et al., 2012), and by turbulent mixing caused by wave action (Lattin et al., 2004).

The density of plastics near the southern California shore was found to be highest near to the bottom, with lowest densities being found in mid-water trawls. Storm events, which increased water turbulence, were found to increase the density of suspended microplastic (Lattin et al., 2004). In the Tamar Estuary, the observed even distribution of high density plastic fragments is believed to be the result of denser particles accumulating near the seabed, where the effects of wind driven mixing are reduced (Browne et al., 2011).

Biofouling also affects a polymer's position in the water column. The buoyancy of polymers enables them to remain in the water column for long periods; however, their surfaces act as settlement sites for a range of species (Saldanha et al., 2003). Build-up of fouling organisms reduces overall buoyancy, causing plastic deposition (Ye and Andrady, 1991).

4.1.4 Settlement

The distribution of deposited plastic is affected by local bathymetry and beach topography. In the Mediterranean, plastic has been observed to collect in areas of slower, deep water (Galgani et al., 1996); the CSA also has regions of slow moving water and a slow flushing time, thought to be up to 4 months, caused by its fjord-like shape (Dooley, 1979; Kasai et al., 1999).

In deeper areas, the residence time of water is increased; here mixing only occurs during the winter when the thermocline is reduced. These sites are more likely to accumulate greater levels of plastic than shallower zones as slower currents cause increased deposition (Browne et al., 2010). The complex bathymetry and circulation of the CSA indicate there should be a high disparity in plastic deposition between different areas.

Due to their shape, local currents and orientation to the wind, certain beaches act as litter sinks (Galgani et al., 2000). Conversely, some beaches do not hold plastic for extended periods. Refloatation of deposited debris results in much of a beach's plastic load being transported to new locations, or remaining in suspension (Williams and Tudor, 2001). The distribution and accumulation of plastics may be similar to that of deposited sediments. Increasing and decreasing depending on local conditions. Unstable, erosional beaches in northeast Brazil were seen to have lower plastic loads than stable beaches (Santos et al., 2009).

Plastics may also be re-suspended by wind driven mixing in near-shore waters, and by trawling in deeper areas (Floderus and Pihl, 1990). This may allow plastics to be redistributed over long periods; therefore, their distribution may be expected to reflect prevailing conditions.

4.1.5 Aims and Objectives

Since their inclusion in the Marine Strategy Framework Directive, there has been an increase in the efforts to establish baselines for microplastic litter throughout the EU. These methods are often based on single sampling events over large

areas, and there is little data available on the short term variability in microplastic distribution. The CSA has a complex bathymetry and circulation pattern, as well as receiving a wide range of plastics from diverse sources. This combination of factors results in large differences in the level and type of debris found between locations. In this chapter the levels of suspended and sedimentary microplastic were determined from sites across the CSA. Plastics recovered were identified both visually and using FTIR analysis, and their origin determined where possible.

4.2 Methods

Benthic sediment and surface water samples were taken from two areas in the CSA, Skelmorlie Bank, the Main Channel (Figure 4.1). These sites corresponded to those sampled for *N. norvegicus* in Chapter Two, and were selected to represent a range of depths and variable distances from known pollution sources. To reduce the impact of tidal state all samples were collected as close to slack water as possible. Depth, sediment type and size, and silt content and the level of floating plastic were recorded for each sampling event.

4.2.1 Suspended Microplastic

Suspended microplastics were sampled using 330 µm mesh plankton tows (Figure 4.2). According to available literature, 333 µm mesh is most commonly used in both manta and bongo net sampling for sampling of microplastics (Hidalgo-Ruz et al., 2012); as a result, our observations will be more comparable with those reported in other areas. After each tow the sample was transferred to individual brevets before being preserved using lugol's iodine. Prior to analysis, the samples were separated using a plankton splitter. Plastics were removed from the first fraction by filtering, then the addition of saturated salt solution to float out plastics. The second fraction was retained as a reference.

4.2.2 Benthic Microplastic

Benthic samples were carried out using two methods. Monthly samples of benthic sediment were retrieved using a Day grab (Figure 4.3), from which a litre of sediment was retained using a beaker. At each location three grabs were carried out to ensure results were not affected by microspatial heterogeneity in plastic distribution. Samples of microplastics at different sediment depths were collected in a once off event in month seven. Sediments were obtained using an adapted corer (Figure 4.4), designed to minimize disturbance of the loose flock layer and prevent compression of sediments. Each core was split into 50cm subsets, for which the level of microplastic was recovered.

4.2.3 Establishing an Accurate Method for Plastic Recovery

To assess the most accurate method for the removal of plastics from sediments, pre-trials of seeded plastic samples were carried out. There have been a number of methods suggested for removing microplastic from sediment, including elutriation, and flotation in super dense solutions. To determine the effectiveness of these methods, plastics were then extracted using filtered seawater, saturated NaCl solution (Hidalgo-Ruz et al., 2012), and LUDOX HS-40 colloidal silica, with a specific gravitiy of 1.3.

Samples were made up using either 0.02g or 0.04g of pre-weathered microplastics, added to 100ml of fine sediment. These were then mixed with 100 ml of the solute and stirred to evenly mix throughout the sample. Containers of varying diameter were used to analyse the effect of sediment depth on sediment floatation. The samples were then placed on a sediment shaker for 20 minutes and the sediment was allowed to settle out over 10 minutes, the solute was then decanted. This was repeated a further four times to assess technique efficiency.

The supernatant was removed and passed through a 63µm filter using distilled water; this removed remaining traces of reagent. The resulting plastics were weighed to determine recovery level. It was found that there was minimal

difference between saturated NaCl and Ludox solution, however reducing the depth of sediment increased overall plastic recovery

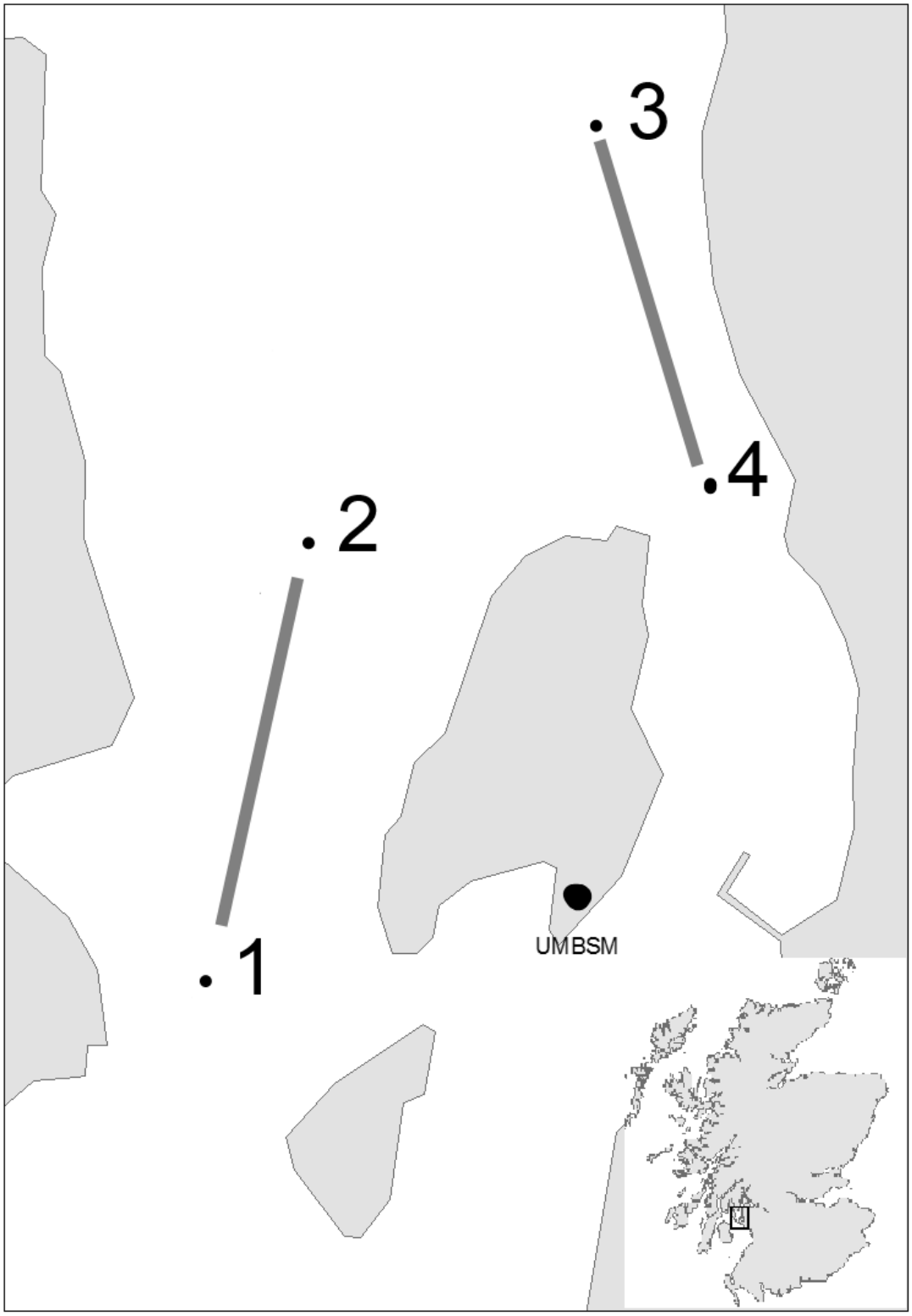


Figure 4.1 Environmental Sampling Sites in the CSA:
T1-2: -4°59.648E, 55°46.233N ~ -4°59.471E, 55°45.639N
T3-4: -4°54.566E, 55°49.835N ~ -4°53.938E, 55°48.463N

Figure 4.2 Retrieving the Plankton Net after a 10 Minute Tow

Figure 4.3 Day Grab used to Collect Monthly Sediment Samples

Figure 4.2 Crabe Corer used in the Collection of Core Samples during Month Seven

4.2.4 Sample Analysis

To determine the sediment composition at each site, each sample was passed through graded sieves, and the water retained and filtered to preserve any silt. The resulting fractions were oven dried before being weighed. The mean grain size was then used to determine the aggregate class as outlined in the Wentworth Scale.

1000ml of sediment, at a depth of approximately 30mm, was mixed with 3000ml of NaCl solution. Samples were then shaken for 20 minutes and floating plastics skimmed off. This was repeated a further three times, and the resulting plastics passed through a 63nm filter. Plastics were counted, and categorized as filaments, fragments, films, and nibs, before being weighed.

4.2.5 Plastic Analysis

To enable comparison between sampling regimes, all recovered plastics were treated in the same manner. In the majority of samples the weight of plastic was too low to accurately record the mass recovered, and the number of items was chosen as the primary methods of assessing microplastic load. Individual microplastics were classified as fibres, fragments, films or nibs and the total of each per sample was recorded.

Of the suspected microplastics, 100 samples were selected at random using a number generator, and subjected to FT-IR analysis as outlined in Chapter Two. The samples were first washed in distilled water and allowed to air dry, before being analysed. The results were then compared to spectra produced by samples of the most commonly recovered plastics.

Another method for the identification of polymers is to examine the way they bend light. In anisotropic materials all molecules will be oriented in the same direction; as a result, light passing through will all be bent at the same angle. Many polymers are classed as isotropic, having the same optical properties in all directions. However, the moulding process results in stress to the polymer

structure causing the polymer chains to line up in the direction of force, resulting in the development of anisotropic characteristics. This is known as stress birefringence. The angel at which a material bends light is dependent on the its refractive index, defined as the speed that light passes through a material as opposed to its speed in a vacuum.

Birefringence is a measurement of anisotropy under polarised light. Light first passes through a polarising lens, which allows only waves oriented in a single direction through. Following this, the polarised light travels through the sample, where it is refracted. This bending of the light beam results in two wave components on different planes. An analyser lens (a second polariser) then reorients the refracted light into an observable interference pattern. The refractive properties of suspected microplastics were also used to confirm the nature of particles not analysed using FT-IR.

4.2.6 Statistical Analysis

The variation in microplastic abundance at each site was statistically analysed in Minitab16. Monthly and spatial variation in the abundance of suspended microplastic was compared using Mann-Whitney U analysis. Monthly and spatial variation in the level of sediment microplastic recovered from each grab site was determined using Kruskal-Wallis analysis.

GLM analysis was used to identify the factors responsible for variation in microplastic abundance. The models were analysed in R (version 3.0.2), and the results were reduced using the stepwise function, subsequently reducing the AIC to identify the model with best fit. Analysis of variance was then used to identify the independent variables which have a significant effect on the abundance of microplastic in the CSA.

4.3 Results

Whilst most sampling took place in good weather, the August event was in heavy seas following a period of high winds and increased wave activity. Data collected by the Met Office indicated that rainfall in the River Clyde catchment was greatest in May, with 124.3 mm, and lowest in March, with only 54.5 mm.

Sediment sampling depths ranged from 61 to 115 m. The sediments recovered in both cores and grabs were all silty, with site average grain sizes between 0.101 mm and 0.283 mm. All sediment and water samples collected were seen to contain plastic. Plankton tows taken over 22 km returned 1036 microplastic items. Of the 30 litres of sediment collected in grabs, (23.4 kg), 944 microplastics were recovered. The results of FT-IR analysis revealed misidentification in 12% of sampled plastics, mainly in the smaller fraction, this was supported by bulk identification using polarised light microscope. There were clear differences in the abundance of the common polymers between the two sites. Floating polymers were mainly made up of PP and PE, with lesser amounts of PS, PVC, N and PES. Sedimentary plastics identified were mainly PES, PP and N, with smaller proportions of PE and PVC (Table. 4.1). Sediment samples had greater proportions of polymer with specific gravity greater than 1.

4.3.1 Suspended Microplastics

The most common type of microplastic recovered was fibres, making up 87.0% of the returned plastic items, followed by fragments (0.6%) and films (0.3%). Comparison of the plastic recovered from the two tow sites was carried out using a Mann-Whitney U test. This indicated a significant difference between the two areas ($W = 666.0$, $P < 0.001$). When examined graphically the variation in microplastic recovered from the Main Channel, site 1, was seen to be lower than that in the Skelmorlie Channel, site 2 (Figure.4.5).

Table 4.1 Polymers recovered from the sediment and water column, as identified under FT-IR analysis

Polymer	Specific Gravity	Source	
		Sediment	Water Column
Polyester	1.38	32 %	8 %
Polyamide	1.13 (N6) - 1.02 (N12)	16 %	6 %
Polyethylene	0.91 (LDPE) - 0.96 (HDPE)	12 %	26 %
Polypropene	0.91	24 %	38 %
P.vinyl Chloride	1.34	4 %	2 %
Polystyrene	0.95 (pre-expansion)	0 %	8 %
Unknown	-	12 %	12 %

The number of microplastic items recovered varied over the course of the year. Highest levels were observed in the Main Channel in August and lowest observed in the Main Channel in February. However, Kruskal-Wallis analysis of monthly variation in recovered microplastic was found to be non-significant (H = 13.25, df = 21, P < 0.900) (Figure 4.6).

GLM analysis comparing location, date, and rainfall indicated that only month had a significant impact upon the level of microplastic in the water column (Table 4.2). This effect was driven by increased microplastic levels in August, coinciding with the earlier mentioned storm conditions. Much of this variation was the result of increases in the main channel, and as a result is not obvious when presented graphically.

Table 4.2 Factors significantly related to the abundance of sediment microplastic in the CSA

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Site	1	8.749	34	137.115	**0.003098**
Month	5	37.949	29	99.166	**3.86E-07**

4.3.2 Horizontal Variation in Sediment Plastic Distribution

In sediment samples, fibres were by far the most numerous plastic type recovered, making up 86% of the sample. Fragments and films were found in only 34% of samples and made up 14% of the total plastic items recovered. Whilst few microplastic fragments were recovered, numerous large plastic fragments and items were revealed via floatation.

Variation in the level of sediment microplastics was also observed between the four sampling locations over the course of the year (Figure 4.7). When analysed using Kruskall-Wallis this was found not to be statistically significant (H = 3.25,

df = 3, P < 0.350). Averaged across the year, site 1 demonstrated the highest plastic contamination, and site 2 the lowest.

Whilst all of the sediment samples recovered contained microplastics, there was considerable monthly variation in the number of items recovered. Analysis using Kruskall-Wallis found this to be statistically significant (H = 16.56, df = 5, P < 0.005) (Figure 4.8). The largest number of microplastics was recovered in August, with spikes in microplastic contamination observed at sites 1 and 3.

Two GLM analyses were used to determine the factors influencing plastic aggregation in surface sediments; the first measured the factors influencing yearly microplastic distribution across the sample area as a whole, the second examined the site specific plastic variation. The factors shown to have the greatest impact on yearly variation in microplastic contamination across the upper Clyde were month, site and sediment size (Table 4.3). Of these, GLM analysis indicated that month had the greatest effect on plastic aggregation. Also statistically significant was sediment size (Figure 4.9), and sampling site.

Table 4.3 Factors significantly related to the abundance of sediment microplastic in the CSA

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Grain Size	1	0.56886	22	1.8149	**7.05E-07**
Silt Content	1	0.00825	21	1.8067	0.5502
Site	3	0.14629	18	1.6603	**0.09676**
Month	5	1.35976	13	0.3006	**2.15E-11**

The second GLM, examining site specific impacts, returned the most appropriate model as depth, sediment size, silt content, month. Similarly to the first GLM, month had the highest statistical significance. This was followed by sediment size and depth, although the latter was only seen to be significant at 95% confidence (Table 4.4).

Table 4.4 Factors significantly related to the abundance of sediment microplastic in the CSA

	df	Deviance	Residual df	Residual Deviance	Pr(>Chi)
Depth	1	404.5	22	9137.5	0.04064
Sediment Size	1	2380.5	21	6757.1	**6.82E-07**
Silt Content	1	66.7	20	6690.3	0.40575
Month	5	5242.6	15	1447.7	**1.80E-10**

4.3.3 Vertical Variation in Sediment Plastic Distribution

As with grabs, fibres were the most regularly returned microplastic. Fragments and films were found in only seven samples. The number of particles found per 50mm segment ranged from 54 to 3, with an average of 19.

Examination of the vertical variation in sediment plastic contamination was carried out using plastics recovered from cores. Long cores were used to examine the difference in microplastic contamination with sediment depth. A Kruskall Wallis test was used to determine changes in plastic contamination with increasing depth, the results of which were found to be non-significant ($x2 = 5.8882$, $df = 6$, $P < 0.4363$).

Analysis of the 2 cm flock layer retained from each core yielded up to eight microplastic items. In order to determine the relationship between bottom water plastics and that of sediment, a spearman's rank correlation was used to compare the level of microplastics in the flock layer with that of the top 50 mm of the core. Test results indicate a weak positive relationship between flock plastic and sediment contamination ($S=145.2604$, $P < 0.742$); however, when results were examined graphically there was no clear correlation (Figure 4.11).

4.3.4 Plastic Contamination and *N. norvegicus*

It has been previously shown that *N. norvegicus* from the CSA aggregate large amounts of plastic compared to other areas. This may be a direct result of contamination of the water column or sediment. In order to examine this, the variation in annual plastic levels and type of microplastics recovered from the environment was compared to that found in *N. norvegicus* in Chapter Two.

The percentage of fragments and films found in the gut contents of *N. norvegicus* were found to be most comparable to those recorded in the sediments, whilst those of the water column were almost seven times as frequent (Table 4.5). The polymer composition of recovered plastic is more closely related to that of the sediment than that found in the water column (Table 4.6).

Table 4.5 Variation in recovered plastics in *N. norvegicus* and the environment

Source	Percentage Samples Containing Fibres	Percentage Samples Containing Fragments and Films
Langoustine	83.1	5.9*
Sediment	100	2.9
Water Column	100	40

Table 4.6 The variation in the percentage of identified polymers recovered from *N. norvegicus* and the environment

		Source	
Polymer	***N. norvegicus***	**Sediment**	**Water Column**
Polyester	37.23%	32%	8%
Polyamide	29.79%	16%	6%
Polyethylene	6.38%	12%	26%
Polypropene	12.77%	24%	38%
Polyvinyl Chloride	1.06%	4%	2%
Polystyrene	0%	0%	8%
Unknown	12.77%	12%	12%

4.4 Discussion

The results presented above indicate a high level of both inter- and intra-site variability in the aggregation of microplastic. In the water column, this was caused by higher abundance of plastic and increased variability in the Main Channel. In sediments, there was limited variation between the four sampled locations; however, site two, at the north end of the Main Channel, exhibited a lower average abundance of plastic on all sampling dates but one. When combining all sampled locations there was significant monthly variation in the level of sediment microplastic, with a significantly higher level exhibited in August. This increase in sediment microplastic corresponded with a period of

storm activity, with high winds and large swell, and a similar spike in the level of suspended microplastic.

The most commonly isolated microplastics from both sediment and the water column were fibres, with low levels of fragments and films. The proportion of samples containing fragments and films was higher in the water column than in sediment, suggesting reduced settlement. However, this may be an artefact of the type of sampling, as a much greater area was sampled during plankton net tows than in sediment grabs. Fibres have previously been seen to be the most abundant form of microplastic in a number of other studies, for example in the western English Channel (Cole et al., 2013), and Irish Sea (Lusher et al., 2014).

FT-IR analysis of the polymer types recovered revealed an 88% success rate in the identification of polymers, with increased misidentification observed in smaller fibres. The main polymer types identified in suspended microplastics were PP and PE, with lesser amounts of PS, PVC, N and PES; this is consistent with polymer types commonly observed in previous studies of floating plastic. The most frequently identified polymers recovered from sediment microplastics were PES, PP and N, with smaller proportions of PE and PVC. The difference in polymer composition is believed to be the result of the different specific gravities of the polymers. Seawater has a specific gravity of 1.025, and polymers with specific gravities higher than this are more prone to sinking. The most commonly identified polymers have numerous industrial and domestic uses, and may have been directly introduced to the CSA or it's originated from within the catchment.

The level of sediment microplastic observed here fits within the range reported within previous studies; however, the proportion of fibres is much greater than that recorded elsewhere (Table 4.7). This may be the result of its close proximity to numerous sources of fibre pollution, such as the collective washing machines of the Clyde catchment, and releases from maritime activities. Similar proportions of fibres have also been recovered from water samples collected from the adjacent Irish Sea (Lusher et al., 2014).

The level of fragments and films is much less than that recovered from the upper Clyde. The higher levels of upstream plastic reported from the upper CSA

are concurrent with other coastal studies, which have linked proximity to sources with increased plastic debris. For example, in the north east Pacific, highest microplastic concentrations were found close to urban areas (Desforges et al., 2014). This is the same as that reported in a number of studies of macro debris, for example, those of Brazilian coastlines (Leite et al., 2014).

Many of the sites at which high levels of plastic were recovered by Zajac et al (unpublished data) appear to be the result of a number of samples from enclosed sea lochs. Previous studies which sampled other enclosed water bodies such as harbours (Claessens et al., 2011) and lagoons (Vianello et al., 2013), have also been seen to return relatively high levels of microplastic debris. As with sediment in suspension, low flow areas can lead to increased deposition (Massel, 1999); following the transport of debris by the incoming current, the residence time is sufficient to enable settlement in the sediments.

4.4.1 Horizontal Variation in Water Column Plastic Distribution

Water column sampling indicated both spatial and temporal variation in level of recovered plastic. The level of microplastics reported here are not as high as those in a number of other coastal areas. However, in the CSA, the sampling locations are relatively open with little obstruction to water flow. High levels of plastic seen in previous studies, such as that in the Queen Charlotte Sound, were thought to be the result of enclosed bathymetry (Desforges et al., 2014).

Another factor affecting the abundance of microplastic in the surface water is salinity. The surface water in the CSA has a low salinity due to the outflow of the River Clyde, which forms an overlying layer of low density, fresh water (Massel, 1999). As the position of plastic in the water column is dependent on its relative density compared to the surrounding water, debris would be expected to sink faster in this low salinity wedge.

Table 4.7 Variation in Recorded Microplastic Debris

Area	Site	Items kg^{-1}	Fibres kg^{-1}	strands as % of total recovered	Author
Clyde	Estuarine	43.8	39.1	88.1	Presented
Clyde	Coastal	180	22.1	12.2	Zajac et al. unpublished
UK	Estuarine	-	35	?	Thompson et al., 2004
UK	Sub-tidal	-	89	?	Thompson et al., 2004
Belgium	Harbours	166.7	66.3	39.8	Claessens et al., 2011
Belgium	Coastal	91.9	59.8	65.1	Claessens et al., 2011
Belgium	Coastal	13	-	-	Cauwenberghe et al., 2013
The Lagoon of Venice	Lagoonal	1445.2	158.9	11	Vianello et al., 2013

The proportion of microplastic fibres in the sample was very high. However, this is unsurprising considering the proximity to numerous potential sources. A number of other studies have shown high levels of fibres; for example, sampling in the north eastern pacific, which resulted in up to 75% fibres, with near-shore samples having the highest proportion of fibre contamination (Desforges et al., 2014).

Transport in near shore, particularly estuarine environments is much more complex than that in offshore environments, where bulk transport is the result of convection, diffusion and wind effects. In addition to the impacts of changing salinity, there is also the effect of complex bathymetry and increased variability in water temperature (Massel, 1999). As a result, high variability may be observed in plastic aggregation in a relatively small area. The results reported here show variability in levels of microplastic recovered at the two trawl sites. The two tow locations differ in their bathymetry and flow rate; site one, in the main channel, is deeper and the surface water travels faster than that at site two (Midgley et al., 2001); as a result, the passage and aggregation of floating plastics might be expected to be variable. It has previously been seen that the distribution of floating plastic is dictated by a combination of environmental factors and the plastic's size, shape and buoyancy.

Those microplastics riding on or slightly above the water's surface are subject to windage, the friction effect of wind on the object's surface (Shaw and Mapes, 1979). The course of an object subject to windage may be very different to one subject to the prevailing current alone. The effect of windage alters subsequent settlement. Rates of litter input on a beach in Tresilian, South Wales have been correlated with wind speed (Williams and Tudor, 2001). Similarly, a study comparing windward and leeward beaches in Curaçao showed that windward beaches exhibited 24.2% more plastic by abundance (Debrot et al., 1999). The degree to which debris is affected is the result of a number of factors; the average buoyancy and shape of debris indicates how much will be above water for wind to affect it (Yoon et al., 2010). When this is considered, the level of variation exhibited by the recovered microplastics is unsurprising given the shape and type of polymers recovered.

4.4.2 Horizontal Variation in Sediment Plastic Distribution

As reported in previous studies, the level of sediment microplastic observed at each site was heterogeneous, varying between and within stations over the course of the sampling period. Averaged across the year, site 1 demonstrated the highest plastic contamination, and site 2 the lowest.

The factors shown to have the greatest impact on yearly variation in sediment microplastic contamination across the Upper Clyde were month, location and sediment size. The monthly variation in the level of sedimentary microplastics was considerable across all sites. The relationship between month and microplastic revealed in the GLM was much higher than that of the other factors. This indicates that the site specific environmental factors are less important than monthly environmental change.

Whilst the variation between locations was not seen to be statistically significant, site specific factors were seen to influence the level of microplastic retention. Of these, sediment size was seen to have the greatest impact, with coarser sediments seen to contain less microplastics. This may be a result of small plastic being more easily released from coarser sediments, as they are more able to move between sediment grains. Finer sediments may also adhere more easily to the surface of plastics, resulting in reduced overall buoyancy. In the study of sediment dynamics, the distribution of particles within the sediment had been seen to influence the energy required for re-suspension. Cubic distribution is defined by the stacking of particles on top of one another; this stacking results in less force being required to dislodge particles. Rhombohedral distribution can be explained as particles nestling between one another, requiring more energy for them to be dislodged (Gray and Elliott, 2009). Fine sediments in which plastic can nestle may be expected to retain more microplastic than coarser, stacking sediments.

One factor not seen to be statistically significant in relation to sediment microplastic was the level of microplastic contamination in the water column. This is perhaps unsurprising as debris recovered from beaches and sub tidal areas has previously shown relationships to windage, as seen in floating plastics. For

example, examination of the number of plastic fragments in the Tamar estuary found larger amounts of plastic at downwind sites, with particles less than 1mm in size demonstrating clearer distribution patterns (Browne et al., 2010). Similarly, offshore movement of debris is determined by geostrophic currents (water flowing between pressure gradients) and the effect of wind, comprised of wind action on the exposed "float", Stokes drift (wind driven waves) and Ekman Currents (Kubota, 1994; Maximenko et al., 2011). However, the effect of windage will have a much smaller impact on plastics riding low in the water column, and may lead to very different distributions.

Dispersal of plastics in the water column is the result of a number of factors, such as the polymer's buoyancy, water turbulence (Lattin et al., 2004), and biofouling resulting in negative buoyancy (Galgani et al., 1996). This will be especially apparent in areas such as the CSA, which are highly stratified. The density of plastics near the southern California shore was found to be highest near to the bottom, with lowest densities being found in mid-water trawls (Lattin et al., 2004). Low proportions and even distribution of high density plastic fragments observed in sediment samples taken from beaches in the Tamar Estuary was believed to be the result of denser particles accumulating near the seabed where the effects of wind driven mixing are reduced (Browne et al., 2010).

Water depth was also found not to significantly influence plastic accumulation; however, this may be the result of comparatively low variation in depth between the four sites. In the Mediterranean, bathymetry and local currents were both seen to affect debris accumulation, with plastic observed to aggregate in slower deep water (Galgani et al., 1996). The complex bathymetry and circulation of the CSA indicate there should be a high disparity in plastic deposition between different areas, which may become apparent should the survey area be expanded.

4.4.3 Monthly Variation in Sediment Plastic Distribution

There are a number of factors that may result in the observed monthly microplastic variation. An obvious impact, highlighted by the August spike in microplastic contamination, is that of abnormal weather events which have a number of associated impacts. Recently, the use of one dimensional column models has indicated that plastic debris is distributed vertically by wind driven mixing, and by turbulent mixing driven by wave action (Kukulka et al., 2012). Storm events, which increased water turbulence, were found to increase the density of suspended microplastic (Lattin et al., 2004).

Increased levels of run off from the land would result in more plastics being released into the Clyde Catchment and increased riverine flows. Associated changes in wave action would result in increased re-suspension and redistribution of microplastics. This would explain the increase in sediment microplastics observed in August. This theory is supported by the (albeit weak – $P < 90\%$) link between rainfall and microplastic shown in the 3rd GLM.

Another potential factor is that of trawling. Data loggers placed on fishing vessels in the CSA by Mars et al., (2002) have shown high levels of trawling in the areas sampled, which have the potential to affect plastic distribution. In areas frequently subjected to trawling effort there may be regular re-suspension of microplastic. The heavy trawl doors agitate the sediment, resulting in a plume of suspended sediment (Churchill, 1989). This agitation has previously been shown to redistribute a range of other pollutants including dissolved pollutants in pore water (Durrieu de Madron et al., 2005) and nutrients (Lykousis and Collins, 2005).

While previously seen to affect only the top few millimetres of sediment (Durrieu de Madron et al., 2005), the large number of trawls carried out monthly in the CSA have the potential to greatly effect sediment dispersal. Sediments re-suspended had previously been seen to be deposited as flocs, before they are dispersed over a wide area (Dellapenna et al., 2006). This repeated re-suspension may also be responsible for homogenizing local plastic distribution,

resulting in the lack of observable variation in microplastic at different sediment depths.

Trawling is also believed to be responsible for the lack of variation observed with increasing depth of sediment and explain the difference observed between flock plastics and those of the upper layer of sediment. However, similar results have been seen in other, less trawled areas. The relationship between depth of sediment and level of plastic was also seen to be statistically non-significant in cores collected off the Belgian coast (Claessens et al., 2011). Analysis of microplastic distribution in the Wadden Sea indicated that fibres have a more homogenous distribution than fragments, spheres and films (Liebezeit and Dubaish, 2012).

4.4.4 The Effectiveness of Environmental Sampling in Long Term Plastic Monitoring

The need to monitor microplastic pollution has been recognized in numerous studies, and included in the recent Marine Strategy Framework Directive. At this time there is little consensus as to the most accurate method of measuring microplastic loads, and numerous techniques and standard reporting systems have been utilized. To date there has been no sequential sampling program of both sediment and water column microplastics, and the results presented above suggest a number of potential pitfalls in monitoring coastal plastic pollution.

Standardisation

Direct sampling of plastic has been used in a range of environments with varying success. Whilst the potential of the surveys for creating a baseline contamination level is not in question, comparability between and even within a technique is often low. Some of this low comparability is the result of inconsistent reporting; however, a number of methods suggested are not conducive to producing comparable results.

Manta trawls, used in numerous studies and fast becoming the forerunner in surface column microplastic sampling, frequently sit partially proud of the water's surface, and skip in heavier seas. Not knowing what proportion of the net opening is passing through the water prevents calculation of the volume of water sampled, thus reducing the comparability of samples to other areas. Perhaps as consequence of this, most results are now presented as items or grams per m or m^2. The impact of which may be less important in offshore habitats, however, in near-shore and estuarine environments the impact of localised currents and tidal flows may have a large effect on water volume sampled. Towing into or away from a strong current may artificially inflate or decrease the number of items recovered in the distance towed.

Another issue highlighted is the variation in plastic at local scales. A number of studies use low numbers of tows, spread over large areas, and as a result may miss much of the variation in microplastic levels.

Variability

Unless covering large areas of open sea, surface water sampling will have limited effectiveness as a measure of microplastic impact. In the open sea most life is in the photic zone, close to floating plastics, or in the benthos, where plastic aggregations are diluted by simple water volume. In near-shore habitats, increasingly complex currents, variation in wind, runoff in heavy rains and mixing related to storm events will cause complex spatial and temporal patterns of microplastic debris. As such, frequent monitoring is necessary to encapsulate this variability.

Sediment plastics will be less prone to daily fluctuations in wind direction and other factors; however the results presented above still indicate monthly changes in the level of microplastic. In order to encompass this variability, numerous samples must be taken throughout the year, avoiding periods of extreme weather.

Sample Contamination

In numerous recent studies there has been a trend in discounting fibres from the analysis, due to possible contamination. During plastic separation and analysis, filter papers were placed in order to assess any airborne microfibers. This revealed low contamination levels, with an average of only 2 fibres collected in 24 hours, and no fragments or films. This low level may be a result of low air movement and minimal levels of traffic through the lab. All results were adjusted accordingly; however, there was minimal effect on the proportions. This study indicates that removal of fibres from analysis may lead to drastic underestimation of microplastic contamination. As fibres have now been observed to be consumed by a range of invertebrate species, this oversight may lead to underestimation of a significant anthropogenic pressure.

Reduction in sample handling would have a corresponding reduction in potential contamination. New techniques to identify plastics within the sediment may provide the answer to this. Recent investigations have been carried out on the usefulness of micro-Fourier-transform infrared spectroscopy in the detection of microplastic seeded in sediment. While it was found to be more effective in the identification of microplastics, the technique has yet to be proven in field samples (Harrison et al., 2012).

4.4.5 Relationships between Environmental Microplastics and those Ingested by *Nephrops norvegicus* in the CSA

As previously indicated, the level of fibre contamination in the CSA is much higher than that reported in other areas; however, when this composition is compared to the plastic recovered from langoustine sampled from the same locations there is a high similarity. Only 2.9% of plastics recovered from sediment were fragments and films, in langoustine only 1.3% of contaminated individuals contained fragments or films. This similarity suggests that the high uptake of fibres in *N. norvegicus* may not be the result of selective uptake, but as a result of the local microplastic composition.

The disparity indicated between the suspended and sediment plastics is to be expected due to their different densities. Comparison of the plastic recovered from langoustine to those recovered from the environment reveals similarities to the composition of polymer types observed in sediments. This suggests that plastics are taken up directly from the sediments or by consuming animals from benthic environments, rather than via consumption of organisms which have previously ingested with microplastic whilst whilst feeding in the water column.

The results presented in Chapter Two revealed that *N. norvegicus* sampled further away from human populations demonstrated a much reduced level of plastic contamination. This suggests that *N. norvegicus* would make a suitable indicator of plastic contamination.

A number of vertebrate indicators have been used as monitors of microplastics. For example surface feeding northern fulmars (Franeker et al., 2004), and shore feeders such as phalarope (Connors and Smith, 1982). However, the difference observed between surface and benthic microplastic in this and other studies, indicates that utilizing such species will not provide an accurate indication of microplastic level beyond that of the surface waters. In addition, the large ranges and uncertainty over the period plastic is retained in the gut of these indicator species suggests that these methods will be subject to a high degree of inaccuracy.

Further offshore, baleen whales have been suggested as possible indicators. Rather than attempting to enumerate stomach plastics, it has been suggested that the level of hydrophobic contaminants such as pthalates should be determined (Fossi et al., 2012). This would then be used as a function of microplastic density. Whilst the sheer volume of water filtered by baleen whales would make this an effective way of monitoring large areas, there are numerous uncertainties associated with this technique. These include the unknown uptake rate of contaminants from plastics to living whales, the variation of these contaminants in both the water column and natural prey, and the availability of samples.

In comparison, the results presented in Chapter One indicate that *N. norvegicus* retain plastic for up to six months in males and one year in ovigerous females, as a result, the variation in monthly plastic levels would be encapsulated in one sampling event, timed to coincide with the moult period. The range of an individual is limited and it can be assumed that any plastic was ingested within a comparatively local area.

4.5 Summary

Suspended microplastic was seen to vary significantly between sampling events and locations. This variation was believed to be the result of variation in riverine microplastic inputs and re-floatation of deposited plastic during storm events. No correlation was observed between sediment plastic loads and those in the surface water.

Sediment microplastic levels were seen to vary significantly between sampling events. Analysis revealed significantly higher levels of microplastic at site two, possibly as result of localised trawling. There was seen to be significant monthly variation in microplastic levels across the CSA. The shape and type of microplastic recovered from sediments of the CSA corresponded to that recovered from *N. norvegicus* sampled from the CSA.

The results of both sediment and surface water sampling indicate that intensive sampling is necessary to accurately encompass fluctuation in microplastic pollution in dynamic environments. Indicator species may be a more suitable indicator of environmental microplastic levels.

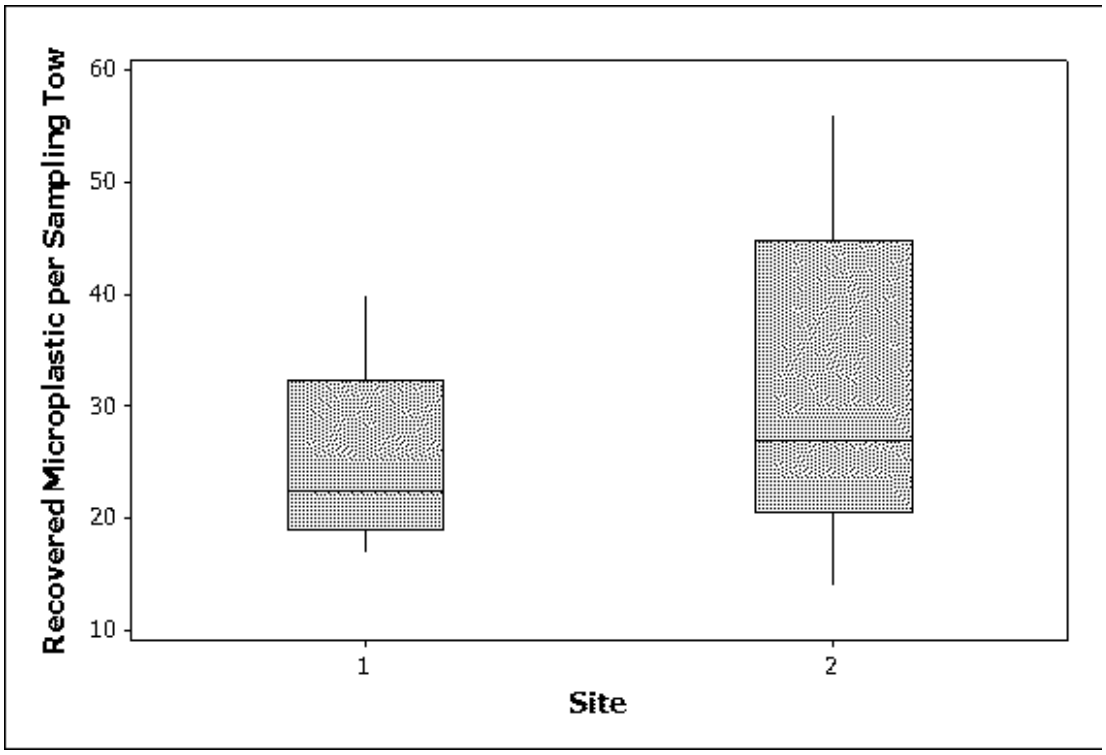


Figure 4.5 The Variation in Recovered Microplastics in Areas One and Two

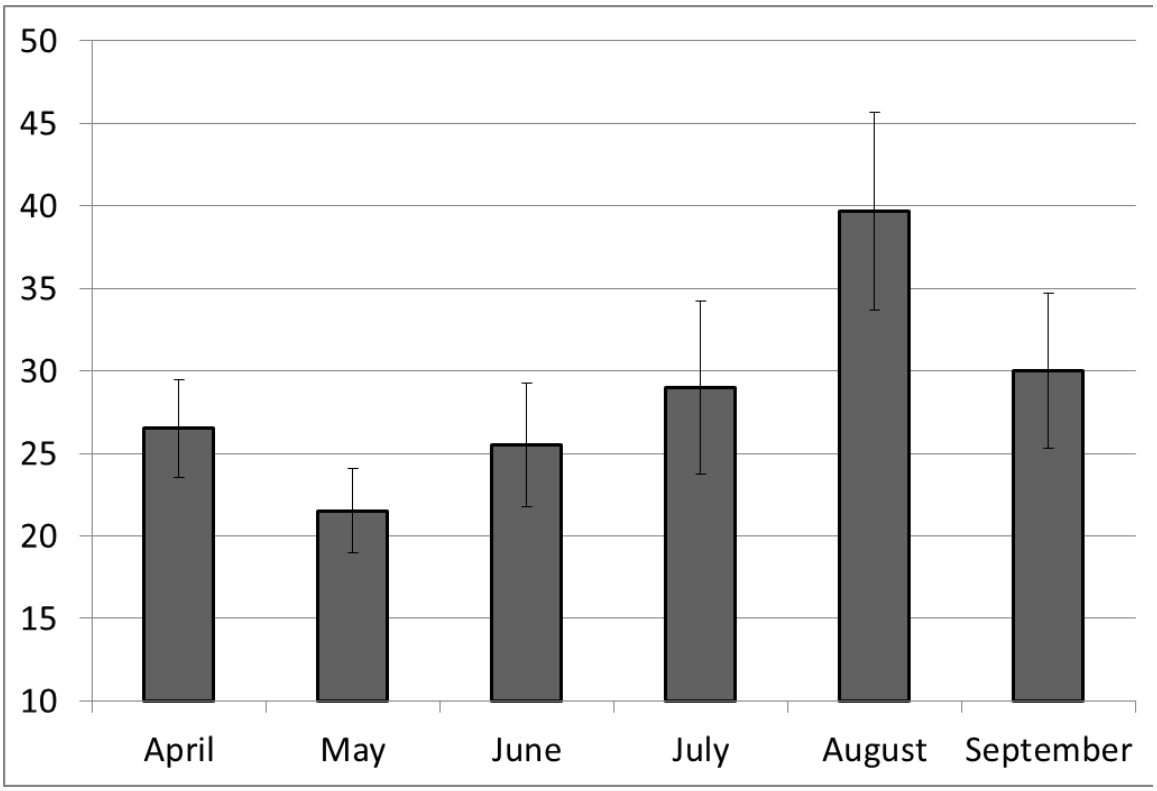


Figure 4.6 Monthly Variation in the Level of Microplastic Recovered from the Water Column

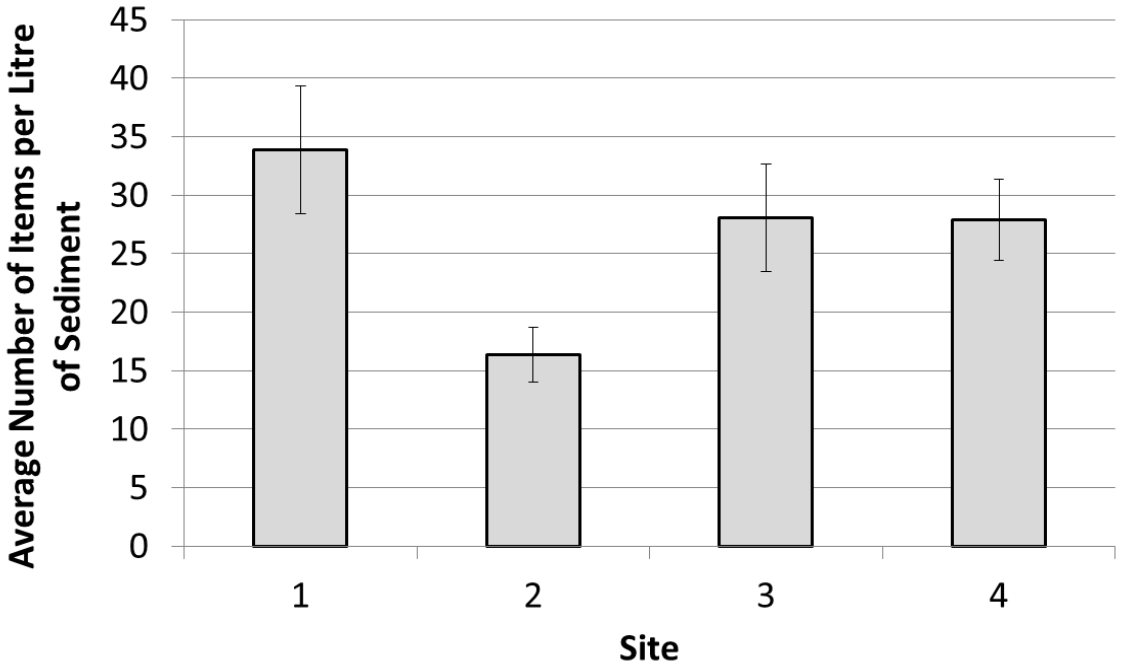


Figure 4.7 Variation in the Mean Number of Recovered Microplastic Items at Each Sampling Station (error bars display standard error)

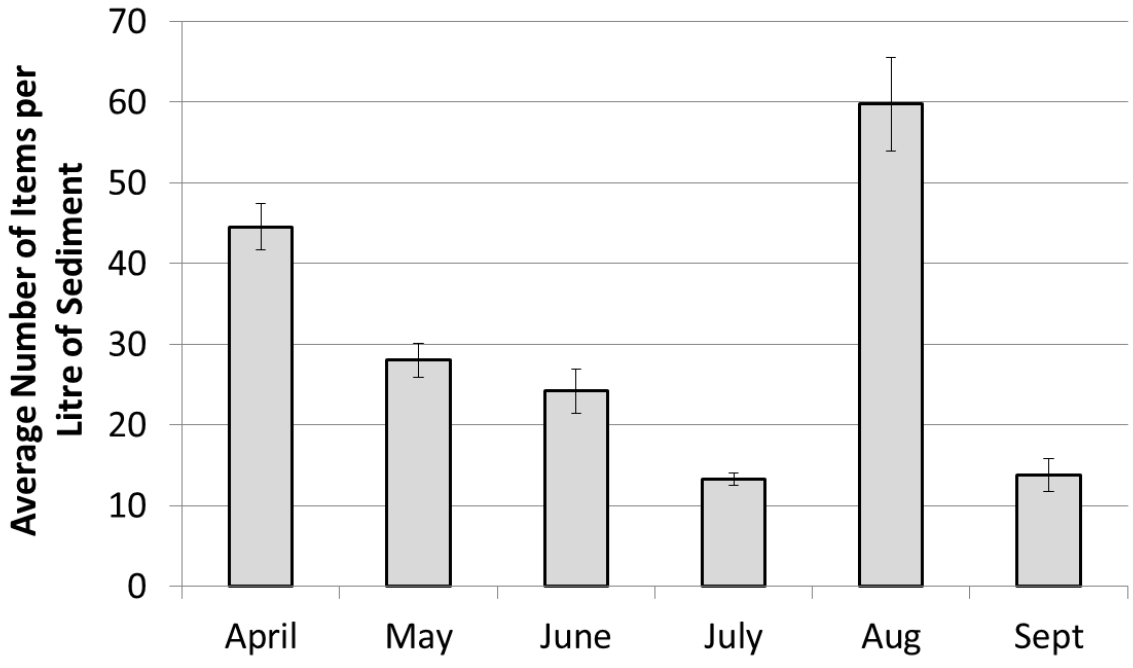


Figure 4.8 Monthly Variation in the Amount of Microplastic Recovered from Sediments (error bars display standard error)

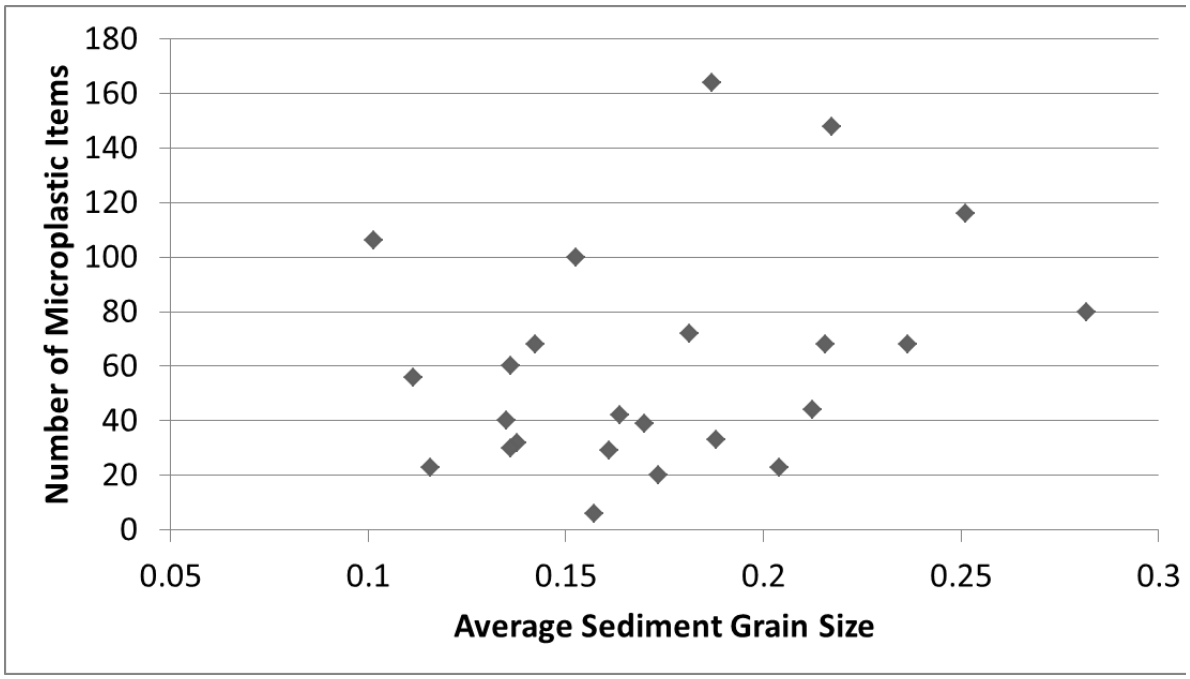


Figure 4.9 The Distribution of Microplastics Recovered with Increasing Sediment Size

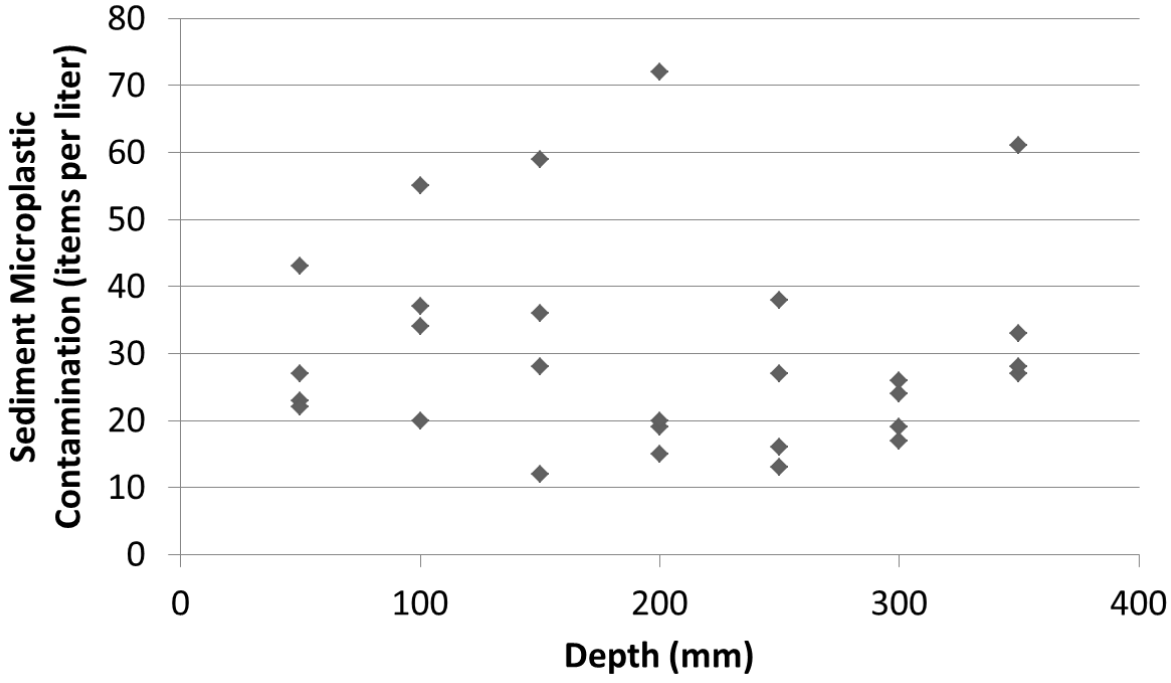


Figure 4.10 Variation Observed in the Level of Microplastic at Increasing Sediment Depth

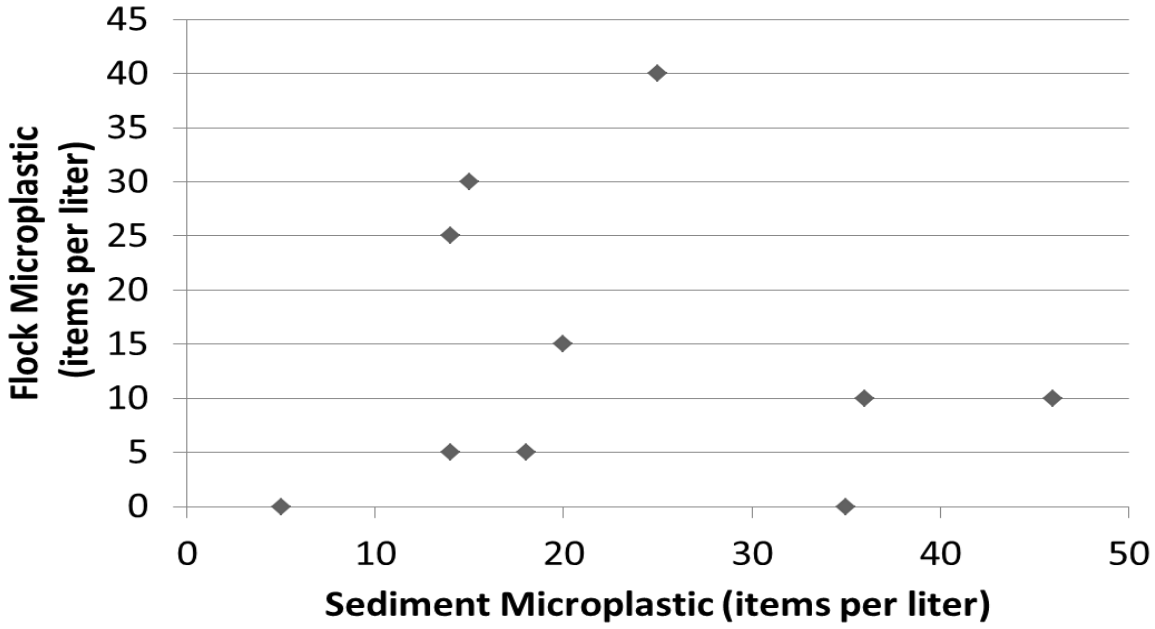


Figure 4.11 The Observed Relationship Between the Levels of Microplastic in the Flock Layer and Surface Sediments

Chapter 5 Gut Anatomy and Feeding in *N. norvegicus*

5.1 Introduction

Ingestion of plastic has been shown to have a range of negative effects on vertebrate species including blockage (Baird and Hooker, 2000) and damage to the gut (Gregory, 2009; Lutz, 1990; Schuyler et al., 2012). Many of the identified impacts, for example false satiation, are increased by increasing mass of consumed plastic (Besseling et al., 2012). Plastic ingestion has only been reported from a handful of invertebrate species, and the potential impact upon the organism is currently unclear.

5.1.1 Plastic Uptake and Feeding in *N. norvegicus*

Pollutants may be accumulated from food or surrounding sediments (Eriksson and Baden, 1998). It has previously been demonstrated that the rate of accumulation is related to feeding. For example, in *N. norvegicus*, ingestion of food contaminated with heavy metals is directly related to raised levels of pollutants in the tissues (Canli and Furness, 1993).

The feeding behaviour of *N. norvegicus* may also be responsible for increased plastic ingestion. *N. norvegicus* feed in a relatively unselective manner. In murky benthic environments reliance on visual cues may cause a reduced ability to distinguish between food and non-food items, resulting in unintentional plastic uptake. Once prey has been located, it is collected by the major chelipeds and walking legs, and either eaten immediately or dragged back to burrows for consumption (Aguzzi and Sardà, 2008). Feeding in this way may allow for plastic to be consumed both with prey and taken up from surrounding sediments.

It has been suggested that *N. norvegicus* are also capable of suspension feeding using the maxillpeds (Loo et al., 1993); raptorial feeding would enable ingestion of suspended microplastic increasing the amount of microplastic available for

consumption. However, at this point suspension feeding in *N. norvegicus* is not thoroughly proven, and its impact on plastic ingestion is in question.

5.1.2 Plastic Accumulation and Gut Morphology

The most frequently observed level of plastic contamination in the previous chapter was large balls of tangled filaments. Inability to routinely egest plastic would increase the mass of plastic carried by an individual, and any related negative impacts of plastic ingestion. Different pollutants can be eliminated via a number of routes. For example, oil pollution can be depurated by many crustaceans (Lavarías et al., 2004; Tarshis, 1981). Also, laboratory experiments have revealed that lugworm, *Arenicola marina*, are able to egest plastics along with food (Besseling et al., 2012), thus reducing their plastic load. However, female Dungeness crabs, *Metacarcinus magister*, have been shown to retain oiled sediments in the stomach throughout a reproductive cycle, resulting in reduced numbers of larvae (Babcock and Karinen, 1988).

For *N. norvegicus* to routinely egest plastic and maintain the observed level of contamination would be highly unlikely. It is therefore believed that *N. norvegicus* retain their plastic aggregations throughout a full moult cycle. It was also observed that larger individuals were less likely to aggregate plastics, indicating that larger individuals are more able to eliminate plastics. One explanation is that the retention of plastics is related to changes in the morphology of the gut related to growth.

The digestive tract of *N. norvegicus* consists of the stomach – which is separated into the cardiac (CS) and pyloric foregut (PS), the mid gut and hind gut (Farmer, 1973). Within the stomach are a series of chitinous teeth known as the gastric mill (GM)(Figure 5.1), which serve to both masticate food particles and distribute digestive enzymes (Phillips et al., 1980). After passing through the gastric mill food enters the midgut, which consists of the hepatopancreas and intestine, it is here that the majority of nutrient absorption takes place (Meziti et al., 2010). This basic morphology is comparable to that in all decapod crustaceans (Castro and Bond-Buckup, 2003; Woods, 1995).

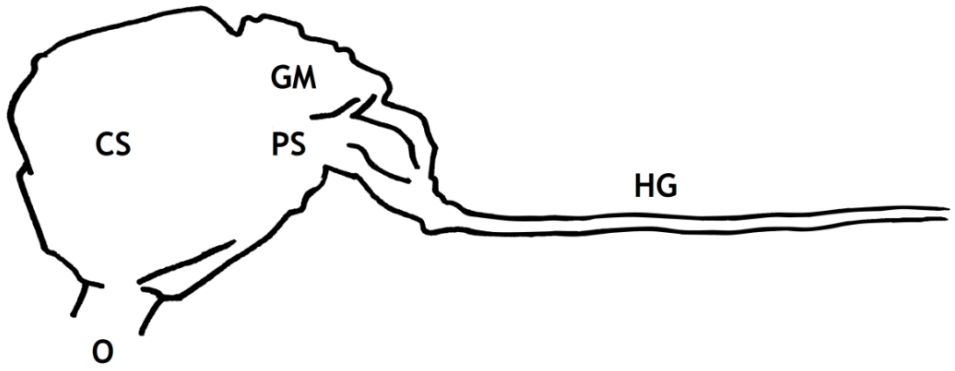


Figure 5.1 *N. norvegicus* Gut Morphology: CS, Cardiac Stomach; GM, Gastric Mill; HG, Hind Gut; O, Oesophagus; PS, Pyloric Stomach

Whilst the expulsion of a small proportion of plastic may occur with undigested food, filaments were observed in the hind guts of only 8 individuals examined. Aggregations of plastic were commonly observed immediately in front of the gastric mill both in the individuals sampled here, and in earlier studies (Figure 5.2) (Murray and Cowie, 2011).

Decapod gastric mills can be highly complex structures. In *N. norvegicus*, the gastric mill consists of ten ossicles, described in Patwardhan (1935). The anterior arch contains the semicircular mesocardic ossicle, articulating with this are the triangular pterocardiac ossicles and the zygocardiac ossicle. The posterior arch is comprised of the pyloric ossicle and connected, four-sided, exopyloric ossicles. Connecting the arches are a further two ossciles; the urocardiac, with a single U-shaped tooth, and the prepyloric; a triangular plate attached to the pyloric ossicle (Figure 5.2). The teeth of the gastric mill are controlled by four types of neurone, activating two antagonistic muscle pairs. Chewing is a two stage process; the two lateral teeth clamp down on food (Figure 5.3.a), after which

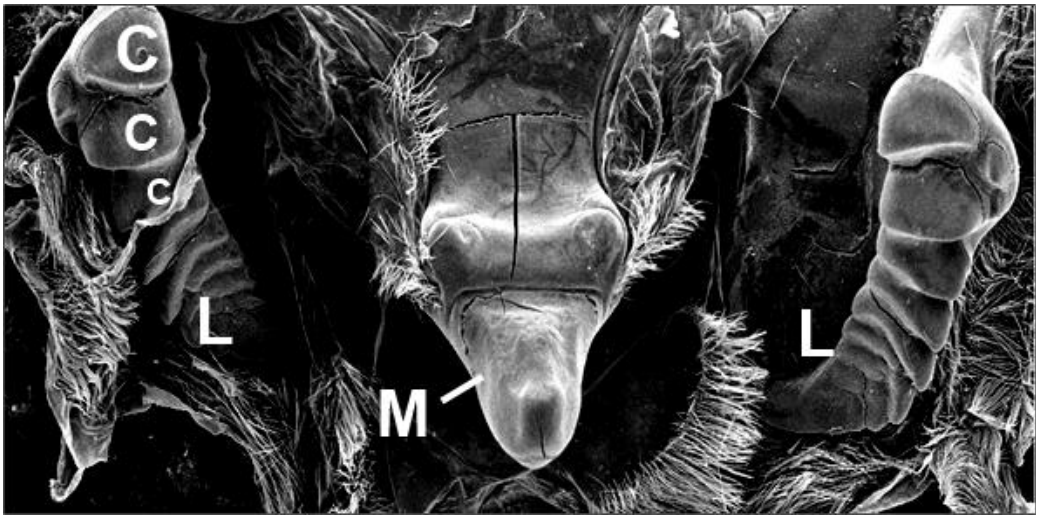


Figure 5.2 SEM image of the *N. norvegicus* gastric mill; L. Lateral Tooth, M. Median Tooth, C. Cusps (only prominent examples labelled)

the medial tooth rasps down and forward over the food (Figure 5.3.b) (Hartline and Maynard, 1975).

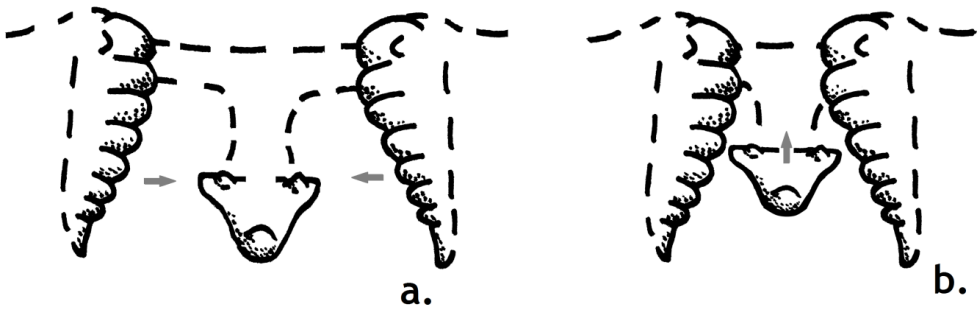


Figure 5.3 a. lateral teeth move together to grip food b. medial tooth rasps toward the hindgut, cutting and moving food

Endoscopic examinations of the gastric mill in *Panulinus interruptus* have shown two types of chewing exhibited by the gastric mill. Squeeze chewing, is characterised as weak contractions of the lateral teeth, and slow movement of

the gastric mill as a whole. In comparison, during cut-and-grind chewing, the lateral and medial teeth move apart to accommodate food, the lateral teeth then close strongly before the medial tooth begins its rasping action (Heinzel, 1988). While this chewing mode may be suitable for mastication of soft bodied or brittle prey, it may not be suitable for the break down and transport of plastics, especially filaments.

In the previous chapter, smaller *N. norvegicus* were seen to accumulate higher levels of plastic. As *N. norvegicus* grow there may be changes in the morphology of the gastric mill which impact an individual's ability to evacuate plastic. Currently, there is little information available on the morphology of the *N. norvegicus* gut in relation to growth. In other Eucarids, such as Euphausiids, the relationship between stomach length and size of the gastric mill and the individual has been observed to be highly variable (Suh and Nemoto, 1988). As such, it is not possible to infer changes in the gastric mill in relation to body size in these groups.

5.1.3 Aims and Objectives

Chapter Two illustrated high levels of plastic aggregation in *N. norvegicus*, most of which were recovered immediately anterior to the gastric mill. In order to determine the impact of gut morphology on the aggregation of microplastic we carried out a morphometric analysis of the structure of the gut and gastric mill in *N. norvegicus* of increasing body size. This data will be used to determine the any changes in gut morphology related to growth, and the extent to which microplastic may be egested by langoustine.

5.2 Methods

5.2.1 Ability of Plastic to Pass the Gastric Mill

In order to determine the ability of *N. norvegicus* to egest plastic, individuals collected from the CSA were starved over a two month period and their plastic

load monitored at monthly intervals. 200 *N. norvegicus* were collected from Skelmorlie Bank. Samples were collected from between 58 and 80 meters using a 70 mm mesh otter trawl deployed from the RV Actinia.

To eliminate potential confounding factors of sex and body size, only males with carapace lengths between 25 and 30mm were selected. Upon landing a third of the catch were immediately frozen, before being dissected and any plastic in their gut removed. The remainder of the catch were held in two tanks, 2.5 m x 1 m x 0.5 m, fed with running seawater. Each month 50 individuals were frozen and dissected; the level of plastic in each group was determined and compared to that of the control group to determine how much plastic was egested. Any individuals which moulted during the two month timeframe were excluded from the analysis and examined separately.

5.2.2 Loss of Plastic during Ecdysis

The gut lining of langoustine is lost during moult. As previously discussed in Chapter Two, at this time there is the potential for plastics aggregations to also be expelled. In order to assess this possibility, a preliminary study of the impact of moult was carried out under laboratory conditions.

N. norvegicus were kept in individual tanks for a two month period. Their diet was 0.5g of squid mantle seeded with five strands of polyproplylene three times per week. After two weeks bilateral eye ablation was performed to induce moulting. The time period between ablation and moult is variable between individuals, however, feeding of seeded plastic continued throughout this intervening period. The moulted fore gut lining and the fresh stomach of each individual were examined for the presence of plastic.

5.2.3 Gut Morphology

Individuals for morphological analysis were collected in two otter trawls, one from Skelmorlie Bank and the other from the Main Channel (full details in Chapter Two). After a starvation period of at least one month to ensure egestion

of all food items, individuals were preserved in ethanol following a week-long starvation period.

Gut Endocasts

Very little is currently known about the changing dimensions of the *N. norvegicus* gut in relation to growth. Endocasting, injecting low viscosity resin into a target organ to produce highly detailed casts, was chosen as a method of determining the stomach volume at increasing carapace length. This method is frequently used to examine the morphology of organs, particularly in the study of the vascular system (Krucker et al., 2006; Lametschwandtner et al., 1990; Northover et al., 1980).

For the purposes of this investigation EpoTek 301 resin (supplied by J.P. Kummer Ltd) was used for endocasting due to its ultra-low viscosity. This resin has also previously been used in the SEM examination of dental microwear in a number of species (Galbany et al., 2004), and has shown a high level of fidelity to original samples (Rose, 1983). Many resins are subject to a certain amount of shrinkage during the curing period, dependent on viscosity and temperature (Krucker et al., 2006); however, the small amounts of resin required to cast *N. norvegicus* guts would produce only a small exotherm resulting in little or no shrinkage (Rose, 1983).

All individuals selected for volume analysis were preserved, and their sex and carapace length recorded as outlined above. Specimens were first preserved in 80% ethanol at room temperature to facilitate the penetration of tissues with fixative and prevent shrinkage (Hayat, 2000; Meyer and Hornickel, 2010). Following fixation for 48 hours specimens were transferred to 70% ethanol (Encarnação and Castro, 2001; Lincoln and Sheals, 1979). Resin casting has previously been seen to result in overestimation of gut volume caused by distension of the gut wall by injected resin (Maller et al., 1983). The process of fixation in ethanol reduces gut elasticity and increases the pressure required to deform the stomach.

A previous attempt to carry out endocasting of the *N. norvegicus* gut was carried out on 37 individuals. In this method the stomach was removed prior to being filled with resin (Wieczorek et al., 1999). However, images of the returned cast reveal a degree of distortion, and it was decided to carry out the casting process without removing the gut, thus retaining its shape. This also assisted in supporting the individual during curing, and enabled the casting of larger groups of individuals simultaneously.

Prior to endocasting, *N. norvegicus* were rinsed with distilled water to remove all traces of ethanol and prevent solvent contamination from affecting the curing process. Individuals then had the pleopods and maxillipeds removed for ease of handling, and the first somite was cut away with scissors to enable the hindgut to be tied off with Nylon line.

Preliminary tests of the endocasting process revealed that airspaces could form during the moulding, to enable resin to reach all parts of the foregut without bubbles forming it was necessary to alter the angle at which held during curing. *N. norvegicus* were first supported at a 45 degree angle, and resin injected via the oesophagus to fill the rear portion of the stomach. After 2 hours *N. norvegicus* were supported horizontally, and further resin injected until it overflowed through the oesophagus ensuring that gut filled with resin (Figure 5.4). To reduce the likelihood of overestimating gut volume by distending the gut tissue resin was injected at a low pressure, using low volume syringes and any excess was allowed to overspill through the oesophagus.

After curing for 24 hours at room temperature the surrounding tissues were scraped away with a scalpel blade followed by sluicing with water (Northover et al., 1980), and the chitinous parts of the gastric mill retained for later analysis resulting in a clean cast (Figure 5.5). To quantify the possible extent of resin shrinkage during curing, tubes of known diameter were also filled with resin. Following the curing period their final diameter was measured and final volume calculated by multiplying the mass of the cast by the resin's specific gravity (Krucker et al., 2006).

Morphology of the Gastric Mill

Examination of the gastric mill by SEM has previously been undertaken in a number of decapods, including *Aegla platensis* (Castro and Bond-Buckup, 2003) and *Notomithrax ursus* (Woods, 1995), and Euphausiids (Suh, 1990; Suh and Nemoto, 1988). In these studies, analysis of gastric mill teeth was carried out by measuring their size and morphology under SEM.

The gastric mill was separated from the gut lining prior to SEM imaging. A triangle of tissue was left to support the ossicles and enable positioning on the stub. Ossicles were then dehydrated in graded ethanol followed by HDMS and mounted on stubs (Allardyce and Linton, 2010).

SEM images were obtained using a Jeol JSM – 5200 scanning microscope. Image analysis was carried out using ImageJ freeware version 1.46. For each individual the length and width of the median tooth, and the length and number of tooth serrations (T) of the lateral teeth were recorded (Figure 5.6).

5.2.3 Statistical Analysis

Data analysis was carried out using Minitab15. The recovered plastic data from the starved *N. norvegicus* demonstrated a non-normal distribution. As a result, the egestion rate data was examined using Kruskall-Wallis test to determine any significant difference in the median level of plastic present. To determine the suitable statistical test to reveal possible relationships between size, gut morphology, the distribution of data was examined using a Kolmogorov-Smirnov test. Following confirmation of normal distribution, the statistical significance of observed changes in the size of the gastric mill with changing carapace length was examined using Pearson's product-moment correlation coefficient (*r*).

The relationship between growth and the structures of the gut was determined by first plotting the log of data and fitting a regression line. The slope and intercept of the trend-line were then used to a general allometric equation:

$$\log y = \log a + b \log x$$

Then translated to the more useful:

$$y = a\, x^{b}$$

a intercept
b slope

Figure 5.2 *N. norvegicus* Supported at 45° during resin curing

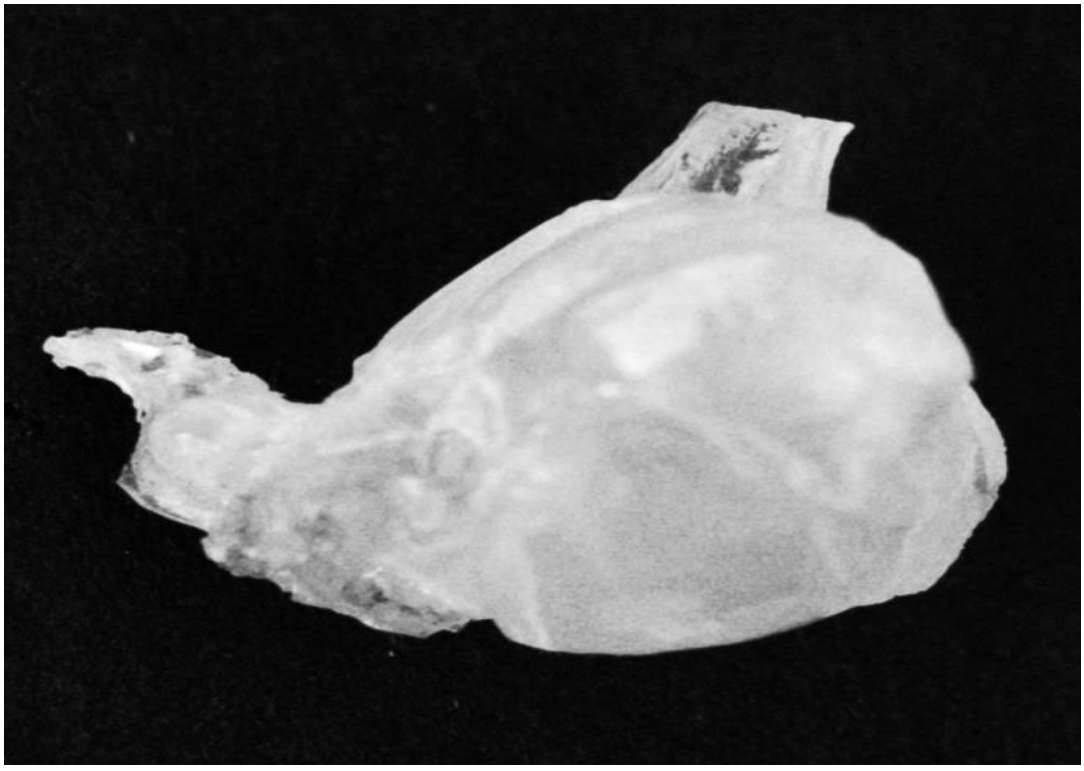


Figure 5.3 Resin Cast of the *N. norvegicus* Stomach; including the oesophagus and cardiac stomach and entrance to the hind gut

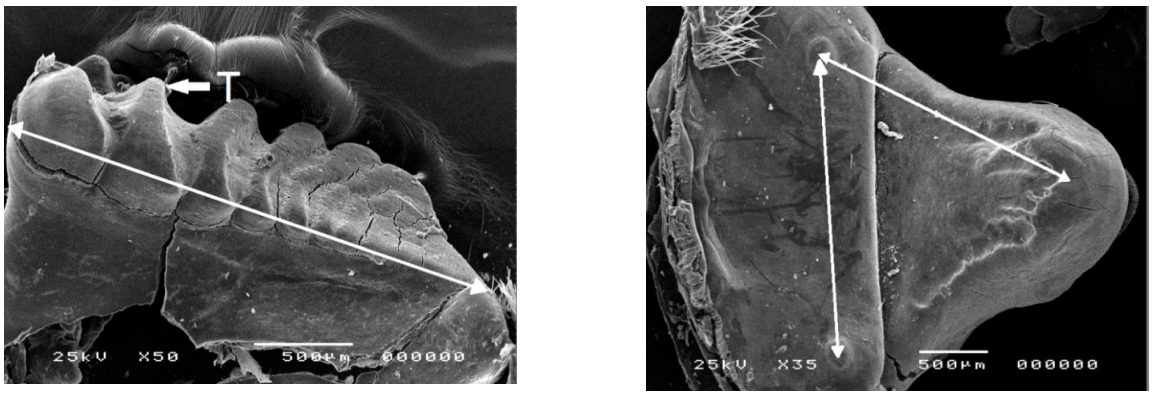


Figure 5.4 Measurement of the Gastric Mill: a) mill tooth T, and length measurement of the lateral teeth, b) length and width of the median tooth

5.3 Results

5.3.1 Egestion of Plastic over time

During the 2 month starvation period 6 individuals died and 3 moulted. Visual analysis of the weight of plastic recovered from individuals at 0, 1 and 2 months indicates a slight reduction in the amount retained in the gut (Figure 5.7). This is possibly due to mechanical breakdown of microplastics in the gut, or by the egestion of smaller fragments. However, the Kruskall-Wallis analysis indicates no significant difference in the median weight plastic retained by *N. norvegicus* over 2 months.

5.3.2 Loss of Plastic during Ecdysis

Of the 10 individuals subjected to eye ablation only 7 moulted within the 2 month time period; of these, 5 individuals had microplastics in the shed fore gut lining. Stomach content analysis carried out on all post moult individuals revealed no remaining plastics in the stomach. The unmoulted individuals were also found to contain plastic aggregations of varying size.

5.3.3 SEM Analysis of the Gastric Mill

Under SEM the teeth of the gastric mill were seen to show varying degrees of wear (Figure 5.8), this affected the ease with which the number of serrations and overall length of the teeth could be recorded. Cracking was also observed on a number of specimens, however, this is likely to be the result of the critical drying process.

Similarly, positive correlations were observed between the carapace length and both the length of the lateral teeth (r = 0.927, $P < 0.001$) (Figure 5.9), and median tooth length (r = 0.902, $P < 0.001$) (Figure 5.10) and width (r = 0.892, $P < 0.001$) (Figure 5.11) in relation to carapace length indicates a strong positive correlation.

Examination of the effects of growth on the morphology of the gastric mill revealed no correlation between the length of the lateral teeth and the number of tooth serrations (r = -0.046, P < 0.585) (Figure 5.12); however, the distance between the 2nd and 3rd serrations of the lateral teeth was seen to increase with increasing carapace length (r = 0.861, P-Value < 0.001) (Figure 5.13).

By fitting regression lines to the available data it was possible to develop allometric equations for the size of both the lateral and median plates of the gastric mill. The line equation derived from the relationship between log carapace length (L_C) and log plate length (L_P) was y = 1.14x + 1.22. Analysis of fit indicated an R^2 of 0.731.

Log Relationship: $\log L_P = -1.22 + (1.14 \times \log L_C)$

Relationship: $L_P = 0.06 \times L_C^{1.135195797}$

The observed relationship plotted between carapace length (L_C) and log plate width (W_P) was y = 1.2346x - 1.6101, R^2 = 0.7962.

Log Relationship: $\log W_P = 1.61 + (1.23 \times \log L_C)$

Relationship: $W_P = 0.025 \times L_C^{1.2346}$

5.3.3 Foregut Volume Analysis

Gut endocasting produced consistently accurate visual representations of the foregut. Statistical analysis of the relationship between carapace length and gut volume indicates a strong positive correlation (r = 0.915, P < 0.000) (Figure 5.14). The average ratio of volume to carapace length was 0.0403 cm^3 per mm. By fitting regression lines to the available data it was possible to develop

allometric equations for the relationship between carapace length (L_C) and gut volume (V_G) demonstrated a line equation: y = 3.1937, x - 4.7811, R^2 = 0.8905

Relationship: log V_G= - 4.7811+ (3.1937 x L_C)

Relationship: V_G= 0.000016553887522 x $L_C^{3.1937}$

5.4 Discussion

5.4.1 Egestion of Plastic by *N. norvegicus*

The high level of plastic recovered in Chapter Two indicated that either plastic was not regularly egested, or that it was constantly taken up from the environment at an improbably high level. The langoustine starved for two months prior to casting showed no significant decrease in plastic contamination. However, of the seven *N. norvegicus* which underwent eye ablation and moulted during a similar two month period, five were seen to expel plastics with the old gut lining. Plastic aggregations were again recovered anterior to the gastric mill.

These factors support the hypothesis laid out in the second chapter, that *N. norvegicus* are able to egest plastic aggregations at moult. This would lead to a different level of plastic aggregation between the sexes, as female *N. norvegicus* moult less frequently than males.

5.4.2 Gastric Mill Morphology and Plastic Retention

The overall morphology of the *N. norvegicus* gastric mill corresponds with that described in earlier studies (Caine, 1975; Factor, 1982; Farmer, 1974; Patwardhan, 1935). The teeth of the mill were surrounded by numerous backward pointing setae. This study is the first to show that growth of the gastric mill is proportionate to overall growth in *N. norvegicus*. Whilst the length of the mill plates increased with size, the morphology of the lateral teeth

showed high variability between individuals. The level of serration was found not to be related to body size, and a number of individuals had different number of serrations on the left and right teeth.

A correlation was observed between the distance from the second to the third serration of the lateral tooth and tooth length. This suggests that the number of serrations does not increase as *N. norvegicus* grow. This is supported by visual comparisons of pre- and post-moult gastric mills. From those individuals where two sets of mills were obtained, it appears that the arrangement of the teeth of the gastric mill varies little from one season to the next. The increase in the size of the mill and its serrations may be necessary in order to manipulate larger prey items, particularly common, hard food items such as chitin and bone. The rate at which food can be egested is also related to the size of the exit to the hind gut. The increase in the size of the gastric mill would increase this, enabling a faster rate of egestion.

In the previous chapter plastic was observed to accumulate at the posterior end of the hind gut, immediately in front of the gastric mill. This may be the result of the dense backward pointing setae directing plastics through the foregut to collect in front of the mill. While the structures of the gastric mill are sufficiently developed to deal with the natural *N. norvegicus* diet (Caine, 1975), the flexible and durable nature of the polymer filaments ingested may prevent their being broken up in the gastric mill.

Unless filaments are oriented parallel to the hind gut and between the serrations of the gastric mill, plastics may not be egested. As plastic would not be degraded further, either by mastication or the action of digestive enzymes, it would remain here until the gut lining was shed at the next moult. Accumulating plastic in this region would result in the aggregations of plastic observed in Chapter Two.

The reduced likelihood of plastic contamination in larger individuals may be the result of changes in the size of the gastric mill. The correlation observed between the distance between serrations and the length of the lateral teeth

suggests that larger individuals would have larger gaps in their gastric mill. This would allow larger pieces of both food and indigestible items to pass from the stomach into the hindgut.

The degree of wear observed on the gastric mill teeth of individuals at intermoult indicates that renewal of the gastric mill is essential in order to maintain feeding efficiency. It may also be the case that reduction in the serration of the lateral teeth would allow greater amounts of plastic to pass into the hind gut. The similarity between morphology of the gastric mill in *N. norvegicus* and that of other decapods indicates that the gastric mill would present an equal barrier to plastic across the order (Caine, 1975; Factor, 1982; Patwardhan, 1935).

Whilst the results indicate that likelihood of plastic accumulation in *N. norvegicus* decreases with increasing body mass, this may not be true of other crustaceans. Evidence from Euphausiid shrimps indicates that the relationship between gastric mill size and carapace length may not be comparable between groups. Gastric mills from representatives from 10 genera were analysed for morphology in relation to feeding strategy. It was found that euphausids have much reduced processes on the ossicles than decapods. For each species studied, an index was calculated based upon the relationship between gut length and the length of the ventral plates; this revealed significant differences in the relationship between gut size and gastric mill between the genera (Suh and Nemoto, 1988).

5.4.3 Gut Volume and Plastic

This chapter represents the first use of two stage endocasting using ultra low viscosity resins to study gut volume. Gut endocasting produced consistently accurate visual representations of the foregut. Statistical analysis of the relationship between carapace length and gut volume indicates a strong positive correlation, and an average ratio of volume to carapace length of 0.0403 cm^3 per mm. Examination of the overall morphology of the *N. norvegicus* gut matches that described in earlier studies (Farmer, 1974).

The results presented here show similarities to those derived from less accurate methods, such as measuring the volume of ingested material (Maller et al., 1983); however, the variation observed was much lower. The gut in decapods is less distensible than that of other crustaceans (Maller et al., 1983), and the absence of anomalous, high gut volumes indicates that there was no distension of the stomach during the casting process. The few proportionally low volumes observed appear to be the result of incomplete casts, usually caused by the presence of minor air bubbles.

Using the relationship between gut volume and carapace length observed here, approximate gut volumes were calculated for the animals examined in Chapter Two. When these were compared with the calculated volume of plastic previously observed the percentage of the gut taken up by plastic was approximately 0.05%. However, due to the inclusion of algae and other materials, and the loose nature of many of the aggregations the actual volume occupied may be much greater.

The proportional increase in gut volume also suggests that larger *N. norvegicus* may be less susceptible to the adverse effects of plastic ingestion. In *N. norvegicus*, there is a constant maximum daily food intake in relation to size (Sardà and Valladares, 1990); therefore the proportion of the gut taken up by food should remain relatively constant. The weight of plastic was observed to decrease with increasing carapace length, and ingested plastic would take up a smaller proportion of the stomach volume. Potential for negative impacts, such as reduced feeding and reduced growth in domestic chickens, *Gallus domesticus,* fed plastics (Ryan, 1988), would be more likely to affect smaller individuals.

Whilst the growth of both the gastric mill and gut volume are correlated with growth, they are not directly proportional to one another. The increase in gut volume is proportionally greater to that of the gastric mill; this is unsurprising as above a certain size the gaps in the gastric mill would reduce its efficiency. However, this may have little or no impact on the accumulation and impact of plastic pollution.

5.4.3 Digestion and the Uptake of Hydrophobic Contaminants

It is known that plastics carry hydrophobic contaminants and additives such as PAHs and bisphenol A (Mato et al., 2001). These have been shown to migrate between the polymer structure and the water column (Mato et al., 2000). The hepatopancreas is responsible for the release of digestive enzymes into the mid-gut, it is these enzymes, along with trituration by the gastric mill which are responsible for the uptake of nutrients (Yonge, 1924). The action of enzymes along with mechanical deformation caused by the gastric mill may result in the release of contaminants (Teuten et al., 2009), which will become available for uptake *N. norvegicus*.

5.4.4 Further Work

The results described above deal solely with post settlement individuals. These results cannot be extended to the larval stages, which exhibit different feeding strategies. Studies of the larval stages of *Homarus americana* have shown that larval lobster stages have less developed gastric mills and longer mid-guts (Factor, 1981).

N. norvegicus zoeae feed mainly on zooplankton such as copepods (Pochelon et al., 2009); it may be that this plankton feeding stage is vulnerable to neustonic plastics which resemble planktonic prey. If this is the case plastics may be ingested by *N. norvegicus* before settlement. As in other decapods, *N. norvegicus* larvae demonstrate simplified gut morphologies and lack the gastric mill (Factor, 1982; Farmer, 1973). This may allow a proportion of ingested plastic to be excreted with other indigestible items. They also show reduced enzyme activity (Kumlu and Jones, 1997; Kurmaly et al., 1990), reducing the likelihood of hydrophobic contaminants migrating from the polymer structure.

Whilst a degree of cracking caused by the dehydration process was observed on a number of samples, the reliability of the results was maintained by omitting those showing significant damage from the analysis. It is believed that these cracks occurred during fixation prior to the casting process. To reduce the

cracking in future studies, individuals should be fixed in more diluted ethanol for a longer period prior to casting.

5.5 Summary

Strong correlations were observed between carapace length and both gut volume, and the size of the gastric mill. Aggregation of plastic directly anterior to the gastric mill may be the result of entrapment by dense, backward pointing setae.

As larger *N. norvegicus* have larger gaps between the serrations of the lateral teeth, they may be able to egest a larger proportion of microplastics; however, this is dependent on filaments being correctly orientated to pass through the mill and along the hind gut. While *N. norvegicus* may be less susceptible to plastic ingestion with increasing size; this may not be true of all decapods, as previous studies have shown differing relationships between gut volume and gastric mill morphology.

The inability of small *N. norvegicus* to egest plastic is supported by the lower plastic weight observed in smaller *N. norvegicus* examined in Chapter Two. The combination of high volumes of ingested plastic and small stomach volume increases the likelihood of false satiation and nutrient dilution effects.

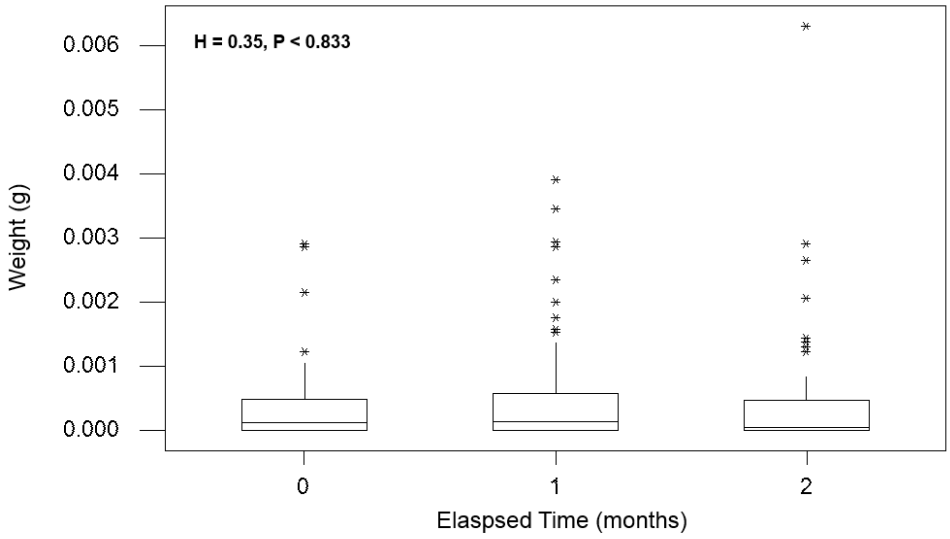
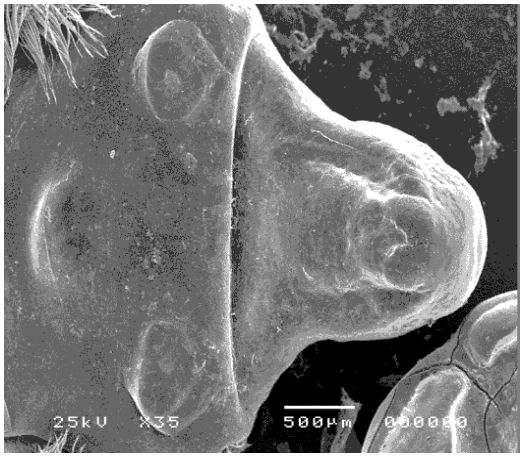
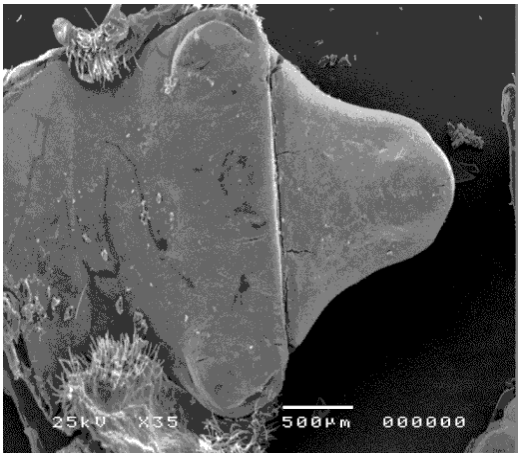


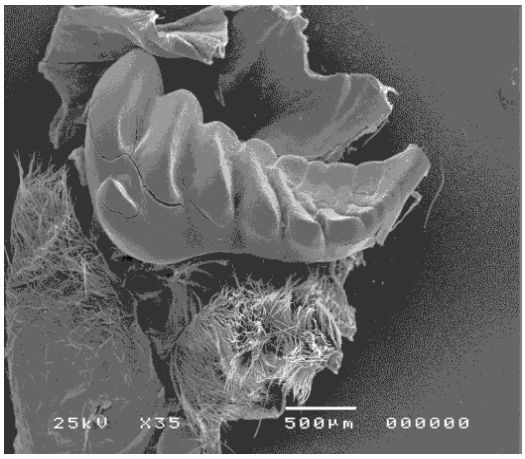
Figure 5.5 Distribution of Plastic Retained by *N. norvegicus* over Two Months



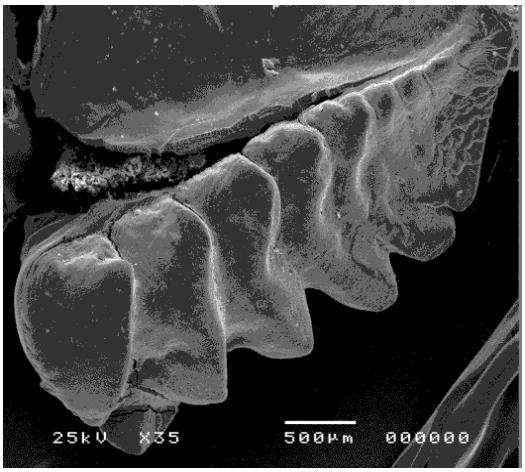
A



B



C



D

Figure 5.6 Varying Degrees of Wear of the Gastric Mill : a – fresh median plate; b – worn median plate; c – fresh lateral plate (some cracking on T1-3); d, worn lateral plate

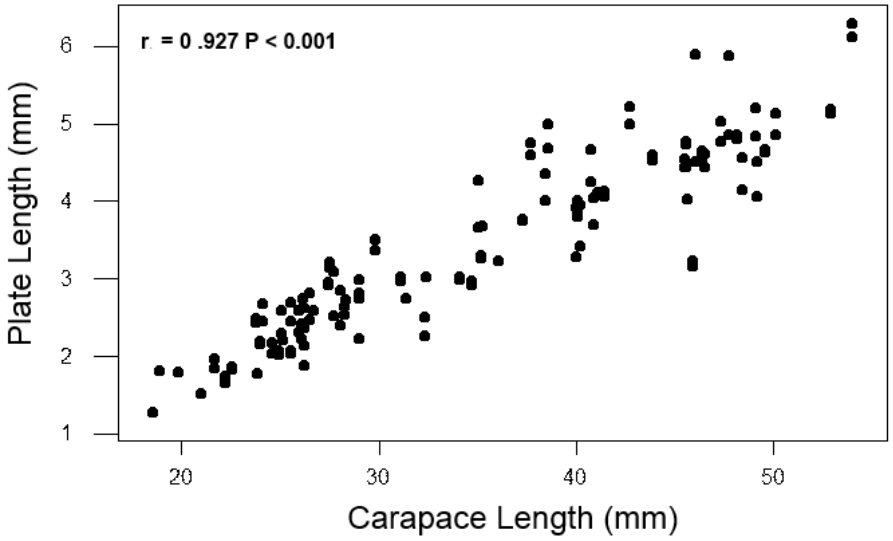


Figure 5.7 Length of Lateral Plate at Increasing Carapace Length

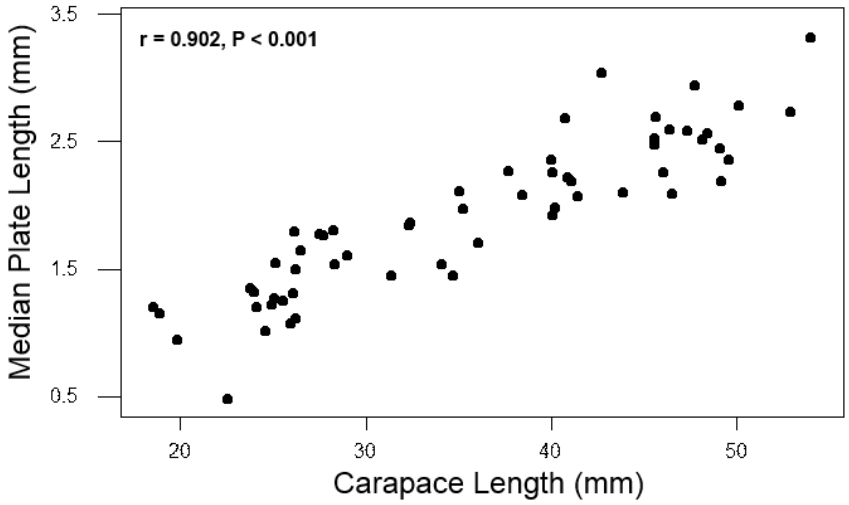


Figure 5.8 Length of Median Plate at Increasing Carapace Length

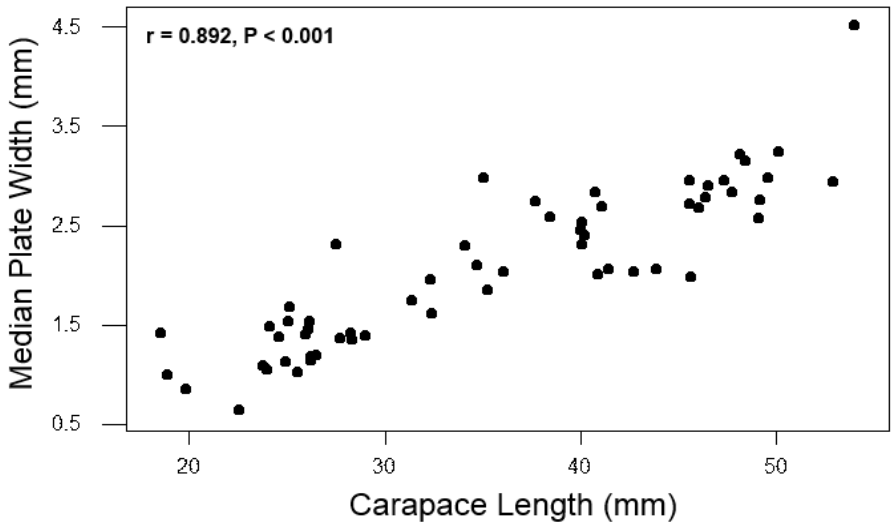


Figure 5.11 Width of Median Plate at Increasing Carapace Length

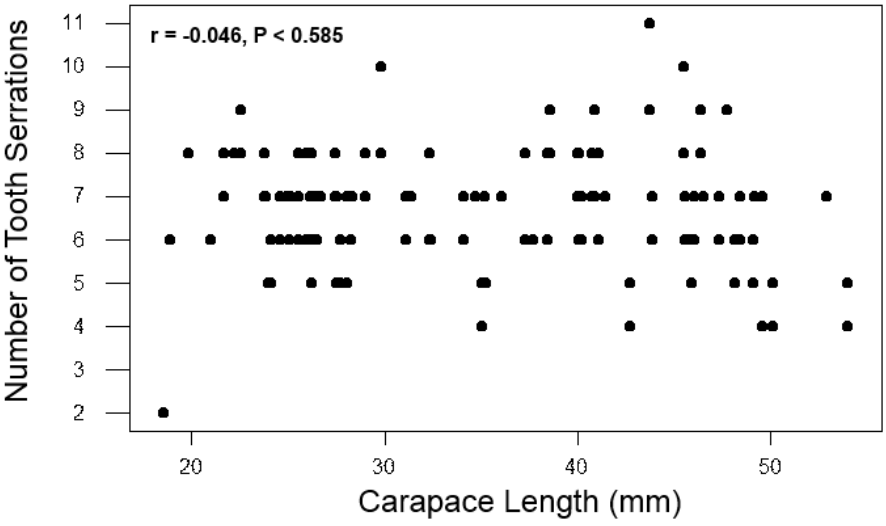


Figure 5.12 Number of Serrations of the Lateral Tooth at Increasing Carapace Length

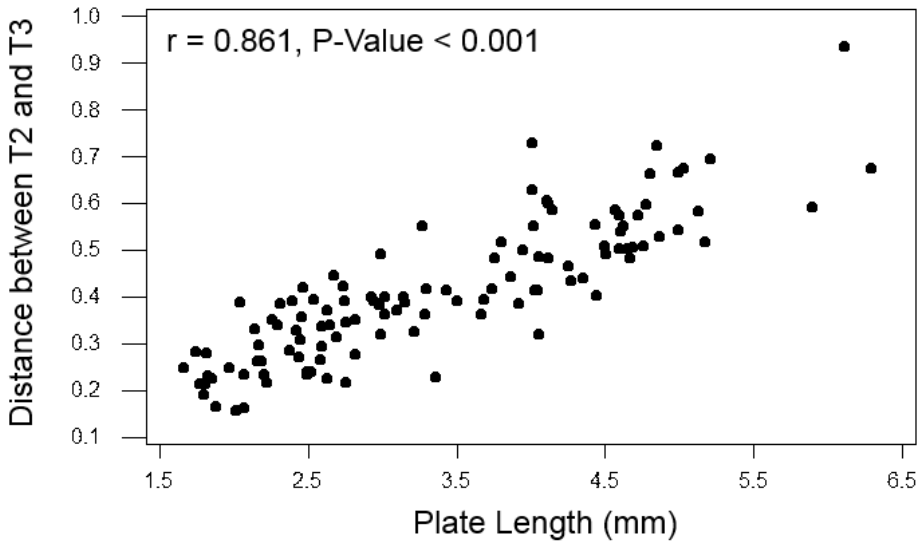


Figure 5.13 Distance between the Serrations at Increasing Plate Length

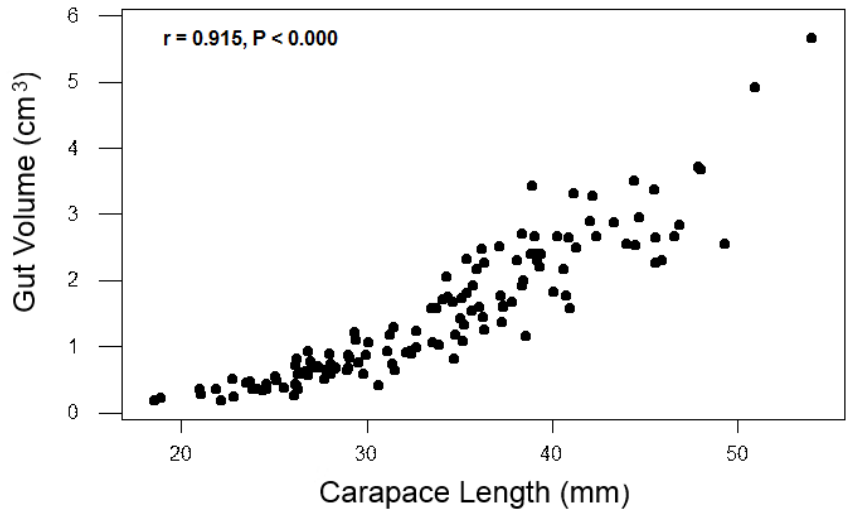


Figure 5.14 Foregut Volume at Increasing Carapace Length

Chapter 6 The Effects of Plastic ingestion on *N. norvegicus*

6.1 The Effects of Plastic Ingestion

Plastic is known to have a range of effects on marine vertebrates, however, few of these impacts have been examined in relation to invertebrates and microorganisms (Cole et al., 2011; Harrison et al., 2011). *Carinus maenus* has been seen to take up plastic from food (Farrell and Nelson, 2013) and directly from the water column via the gills (Watts et al., 2014b). The analysis of langoustine gut content reported in Chapter Two shows plastic uptake in 84.1% of *N. norvegicus* sampled from the CSA. The regular occurrence of large plastic aggregations indicates that plastic is readily ingested; as a result, *N. norvegicus* are an ideal subject for the examination of long term impacts of plastic ingestion on invertebrates. In this chapter the impact of plastic ingestion on *N. norvegicus* is examined by means of a long term exposure study.

6.1.1 Gut Damage and Impaired Nutrient Uptake

Unlike traditional POPs, most microplastics are too large to be absorbed into the body, remaining in the gastro-intestinal tract. These plastic aggregations may have a range of direct impacts on the gut (Baird and Hooker, 2000; Boerger et al., 2010; Gregory, 2009; van Franeker and Bell, 1988). Plastic may abrade or pierce the gut lining, resulting in swelling and increased chance of infection (Gregory, 2009). Aggregations of plastic have also been seen to block the digestive tracts of vertebrates, inhibiting the consumption, digestion and subsequent excretion of food (Baird and Hooker, 2000; FDoNR, 1985) .

Plastics remaining in the gut may result in false satiation, a reduction in feeding as a result of a portion of the gut volume remaining full (Ryan, 1988). This has been observed in the lugworm, *Arenicola marina*, which exhibited a reduced feeding rate when exposed to polystyrene-seeded (7.4%) food (Besseling et al., 2012). Chickens fed plastics were seen to exhibit lower rates of feeding and reduced nutrient uptake (Ryan, 1988). Similarly, examination of plastic load and

body mass in flesh-footed shearwaters, *Puffinus carneipes*, recovered from Lowe Howe Island indicated a reduced body condition in relation to plastic load (Lavers et al., 2014). However, the retention of plastics, and their ability to cause false satiation may vary between species. For example, white chinned petrels, *Procellaria aequinoctialis*, fed plastic demonstrated no reduction in either nutritional state or body condition (Ryan and Jackson, 1987).

Damage, blockage and false satiation may all result in nutrient dilution, a reduction in the effective uptake of food by an organism (Ryan, 1988). Chronic nutrient dilution, caused by frequent or continuous exposure to microplastics, may result in alterations in body condition similar to that resulting from long periods of starvation. Under starvation conditions animals utilise energy stores in order to maintain respiration (Sánchez-Paz et al., 2006). This has previously been observed in green and loggerhead turtles. In a repeated feeding experiment, individuals fed latex and plastic showed a decrease in blood glucose for up to 9 days following feeding (Lutz, 1990).

The plastic retention observed in *N. norvegicus* (Murray and Cowie, 2011) and other crustaceans (Farrell and Nelson, 2013; Katsanevakis et al., 2007) may result in a number of biological effects similar to those observed in vertebrates. The effects of which may be observed as reduction in an individual's fitness or growth rate, or an increase in mortality.

6.1.2 Additives and Contaminants

The risk of uptake of both plastic additives and hydrophobic contaminants adsorbed from sea water was briefly discussed in Chapter One. While many potentially harmful additives are no longer used in the manufacturing process, the age of much of our plastic debris means that their effects remain relevant.

Microplastics sampled worldwide have shown varying levels of hydrophobic contaminants. In the Mediterranean, microplastic particles have shown uptake of a number of phthalates (Fossi et al., 2012), however, these plastics were not separated from other debris. More robustly, analysis of resin pellets sampled

from both the Japanese Pacific coast and the Sea of Japan indicated varying levels of DDT, DDE, and nonylphenol (Mato et al., 2001). The most comprehensive list of adsorbed contaminants has been compiled by the International Pellet Watch project, which monitors globally acquired pellets for the presence of a range of persistent organic pollutants (Ogata et al., 2009). The most commonly isolated contaminants are PCBs, DDTs and HCHs.

Some areas are more at risk of the impact of adsorbed contaminants than others. Horizontally, regions of high industrial activity have been shown to exhibit contamination levels of 1 -3 orders of magnitude higher then remote areas (Heskett et al., 2012).Vertically, models of the portioning of hydrophobic contaminants also indicate that plastics will draw down contaminants into benthic environs (Teuten et al., 2009), increasing the risk to a range of bottom dwelling species, including *N. norvegicus*.

At this time, the relationship between microplastic uptake and that of hydrophobic contaminants has been observed in only a handful of species. Analysis of the levels of PCBs, DDTs and dieldrin in great shearwaters, Puffinus gravis, indicated that only PCBs were positively correlated with the amount of plastic consumed (Ryan et al., 1988). However, the results may be confounded by variation of chemicals in the shearwater's regular diet. In laboratory experiments, PCB loads in the tissues of the lugworm, Arenicola marina, were seen to increase by between 1.1 and 3.6 after being exposed to sediments seeded with polystyrene microspheres (Besseling et al., 2012).

Despite usually being considered a threat only to small animals, larger organisms may be at risk from hydrophobic molecules carried by microplastics. Examination of the levels of phthalates in the water column, adsorbed onto microplastics, and in tissue samples recovered from fin whales, *Balaenoptera physalus*, in the Mediterranean indicate that microplastics may be an uptake route for such contaminants (Fossi et al., 2012), however this has yet to be directly demonstrated.

Invertebrates are frequently used as indicators for a range of anthropogenic impacts (Koop et al., 2011), and their responses to a range of chemical stressors,

including those found in plastic debris, have been widely studied. Examination of PCB contamination in shrimp (van der Oost et al., 1988) and crabs showed aggregation of POP congeners both from contaminated sediments and through the food chain (Porte and Albaigés, 1993). DDT, DDE and PCB concentrations in shrimp, *Parapaneus kerathurus*, from the eastern Mediterranean coast closely resembled that of surrounding sediments, whereas concentration observed in fish was considerably higher (Bastürk et al., 1980). Similar results were seen in velvet swimming crabs, *Necora puber*, and *N. norvegicus* sampled from Brittany and Normandy (Bodin et al., 2007).

Examination of the haemotoxic effects of PCBs on common shrimp, *Crangon crangon*, showed a decrease in haemocyte count and overall volume (Smith and Johnston, 1992). PAHs are thought to affect the reproductive success of copepods (Wirth et al., 1998). Phenanthrene, a PAH used in plastic production, has been seen to taken up by *N. norvegicus* (Palmork and Solbakken, 1979). Monitoring the uptake and elimination of radiolabelled phenanthrene was observed from a range of tissue groups. Highest levels of accumulation were observed in the hepatopancreas and muscle tissue (Palmork and Solbakken, 1980). Subsequent observations have shown that phenanthrene can be metabolised by *N. norvegicus*; however, this process is much slower in the hepatopancreas, with fewer metabolites, such as hydroxyphenanthrene, seen here than in the gonads and intestine. This may be the result of the formation of vacuoles, observed to take in contaminants (Solbakken and Palmork, 1981).

The accumulation of hydrophobic contaminants and plastic additives by benthic invertebrates may be related to the level of contamination of the surrounding sediments. The accrual of PAH loads has been seen to be the result of complex relationships between the level of contamination of the surrounding water and the quantity of food consumed (Baumard et al., 1998a; Baumard et al., 1998b). A review of available data on the rate accumulation in numerous exploited marine species indicated that crustacean tissues had the highest concentrations of PCB; contamination level was seen to vary with location and position in trophic level (Domingo and Bocio, 2007).

Microplastics may provide an additional route for the uptake of these hydrophobic compounds. In *N. norvegicus*, the uptake of contaminants, such as heavy metals, is known to vary depending on season and sex (Canli and Furness, 1993). It is believed that the ingestion of contaminated microplastics may result in leaching of chemicals into the digestive juices, and subsequent uptake by the individual. The distribution of contaminants may vary between tissue groups; adsorbed heavy metals have been observed to be accumulated in differing amounts between tissue groups (Canli and Furness, 1993).

6.1.3 Identifying the Impacts of Plastic Ingestion in *Nephrops norvegicus*

The most commonly observed impact of plastic pollution is digestive impairment, either by false satiation or nutrient dilution. Such impairment would result in decreased nutritional uptake and, in acute cases, starvation. Reduced nutrient availability, either by controlled starvation experiments or seasonal variation in food availability, has previously been shown to result in a number of observable changes in crustacean physiology.

In the early stages of nutritional stress, *N. norvegicus* regulate energy demands by way of metabolic depression (Parslow-Williams et al., 2002; Watts et al., 2014a). Change in metabolic rate has also been observed in numerous species exposed to a range of stressors. Rising water temperature has been seen to affect metabolic rate in *Jasus edwardsii*, this was observed as a steady increase in oxygen consumption up to the thermal limit at 24ºC, at which there was a marked reduction (Thomas et al., 2000). Increases have previously been observed in the metabolic rate of *H. americanus*, which was seen to double when exposed to low salinity (Jury et al., 1994). This reduction in metabolic rate causes a decrease in the individual's energy demands, thus slowing the catabolism of energy reserves, such as lipids (Storey, 1988).

Despite this reduced metabolic demand, reliance on energy stores over long periods would lead to depletion of storage molecules such as lipids, causing an increase in their metabolites (Watts et al., 2014a). The reduction in energy

reserves lead to observable changes in biochemistry and morphology which may be monitored as indices of nutritional distress. These indices range from monitoring changes in mass and density of specific tissues, to highly sensitive molecular methods.

Composition of Haemolymph

Crustacean haemolymph is comprised of water, salts, and organic compounds, and carries haemocytes, which perform a range of functions (Johansson et al., 2000). Haemocyanin, a copper containing metalloprotein responsible for oxygen transport, is the most common organic compound in the haemolymph (Depledge and Bjerregaard, 1989). Other organic compounds include a range of proteins, many of which are responsible for immune responses (Ai et al., 2004; Fredrick and Ravichandran, 2012).

Seasonal variations in food availability (McAllen et al., 2005), and controlled starvation experiments have been shown to result in decreased concentrations of proteins in the haemolymph (Djangmah, 1970; Stewart et al., 1972; Uglow, 1969). For example, starvation experiments have demonstrated decreases in total blood protein in the western rock lobster, *Panulirus longipes* (Dall, 1974), and an increase in the rate of haemocyanin breakdown in a number of other decapod crustaceans (Barden, 1994; Hagerman, 1983; Stewart et al., 1972). Metabolic depression also results in changes in the structure and concentration of a number of regulatory enzymes (Storey and Storey, 1990). Monitoring such changes is non-destructive and can be carried out prior to and following plastic exposure.

Hepatopancreas Copper

The hepatopancreas, often referred to as the mid-gut gland, is responsible for the formation of digestive enzymes and uptake of nutrients (Ceccaldi, 1989; Vonk, 1960), as well as the synthesis of haemocyanin (Senkbeil and Wriston Jr, 1981). The accelerated breakdown of haemocyanin described in the previous section, results in the release of copper – two atoms per molecule of

haemocyanin. This excess copper is thought to be taken up by the hepatopancreas (Barden, 1994; Taylor and Anstiss, 1999; Watts et al., 2014a).

In *Crangon vulgaris*, starvation and breakdown of blood protein was seen to result in an increased concentration of copper within the hepatopancreas (from 82µg to 3177µg per gram of dry tissue)(Djangmah, 1970). Similarly, simultaneous monitoring of haemolymph and hepatopancreas copper concentrations in *N. norvegicus* have demonstrated significant differences between starved and fed individuals (Watts et al., 2014a).

Hepatosomatic Index

Energy storage molecules such as triglycerides and also glycogen are also used to monitor an individual's nutritional health (Koop et al., 2011). During extended periods of starvation, crustacea will utilize a range of energy stores. Under normal circumstances, this process begins with glycogen, followed by lipids, and finally proteins (Sánchez-Paz et al., 2006). Both lipids and glycogen are stored in the hepatopancreas (Farmer, 1975). Reductions in levels of stored glycogen in both the hepatopancreas and muscle tissues have previously been related to an induced starved state (Barden, 1994).

In the southern rock lobster, *Jasus edwardsii*, starvation over 14 and 28 day periods resulted in decreased lipid concentrations, first in the hepatopancreas, then the tail muscle (McLeod et al., 2004). Similar results were observed in the American lobster, *Homarus americanus*, which exhibited decreased concentrations of both lipids and stored glycogen in the hepatopancreas after starvation periods up to 8 months (Stewart et al., 1972); and again in *N. norvegicus*, in which reduction in lipid and increased water content were observed in the hepatopancreas and tail muscle after 12 weeks (Watts et al., 2014a).

Similar responses to starvation have also been recorded in decapod larval stages. *H. americanus* larvae were monitored for alteration in moult cycle and changes in hepatopancreas during periods of starvation. Starved individuals demonstrated reduced lipid content of hepatopancreatic R-cells, and decreased development

until a marked “point of no return”. At this point lipid levels were thought to have decreased to a level at which they could not be recovered (Anger et al., 1985).

Changes in hepatopancreas composition may be measured in a number of ways. Decreases in the total lipid content in the hepatopancreas are associated with an increase in water content, as well as an overall reduction of hepatopancreatic mass (Anger et al., 1985; Watts et al., 2014a). A second measure, Hepatosomatic Index (HSI), calculated as the weight of the hepatopancreas as a proportion of overall body weight, is frequently used as a measure of nutritional health (Jones and Obst, 2000). For the purposes of this study both HSI and hepatopancreas water content were used.

Body Mass

One long term monitor of the effects of reduced nutrient uptake is growth. Growth in crustaceans occurs through a process of successive moults. During this period the carapace is shed to reveal a soft exoskeleton. This allows newly moulted individuals to absorb water and to increase their body mass before the new carapace calcifies (Ingle, 1995; Wang et al., 2003). Nutritional state also influences the moult process, with starvation resulting in delays in the transition between subsequent instars (Anger et al., 1985). Due to the long periods between moults in many invertebrate species the frequency of recordings is limited. However, variation in tissue density throughout the intermoult period may be recorded as a change in mass.

6.1.4 Aims and Objectives

The level of plastic ingestion observed in Chapter Two, and its accumulation within the foregut observed in Chapter Five indicate a high risk of impaired digestive efficiency in *N. norvegicus* which have consumed plastic. This chapter aims to identify negative impacts of plastic ingestion on *N. norvegicus*. In order to achieve this, changes in a range of measures of body condition were monitored in relation to plastic contamination. In addition, changes in feeding

amount and rate were examined to determine any false satiation affect which may be related to plastic retention.

6.2 Methods

6.2.1 *N. norvegicus* Collection and Management

N. norvegicus were sampled from the Main Channel of the CSA in otter trawls from the RV Actinia on the 12/02/2013. Any obviously weak or damaged individuals were discarded. Many of the selected indices can also be influenced by confounding impacts such as, moult stage, hypoxia (Lorenzon et al., 2011), and ovary maturation in females (Aiken and Waddy, 1992; Lorenzon et al., 2011); as a result, recently moulted males were selected for this study.

As it is believed that *N. norvegicus* are able to egest plastics at moult individuals were sampled prior to the moulting period. Upon landing undamaged individuals were transferred to a holding tank for a month long period to allow any weakened individuals to be removed and remaining individuals to complete their moult cycle.

After this time, individuals with carapace lengths between 20 and 30mm were randomly separated into plastic fed treatment (Group A) and fed control (Group B) and unfed control (Group C) groups. At month 0 individuals were measured and weighed and approximately 50 µl of haemolymph taken from the pericardium using disposable syringes fitted with 22 gauge needles. Haemolymph samples were immediately tested for protein concentration (described below).

N. norvegicus were then transferred to individual tanks fed by separate supplies from a semi-open sea water system, and allowed to acclimatize over 30 days. No burrowing substrate was provided to prevent extra plastics being introduced to the tank system. During the treatment period both groups A and B were fed 1.5 grams of fish per individual twice weekly and group C were starved. Twice a week group A were fed fish seeded with 5 strands of polypropylene, group B were fed “clean” fish. After 8 months a second sample of haemolymph was

taken and examined for protein concentration. Individuals were measured and re-weighed, prior to dissection. The gut of each individual was transferred to 80% ethanol for analysis of plastic contamination, and the hepatopancreas removed and stored at -80C.

6.2.2 Feeding Rate

The amount of food consumed was examined for groups A and B. The standard ration of 1.5g of squid mantle was added to tanks and animals left for periods of 6 and 24 hours. After 6 hours, food was removed and reweighed to determine the initial weight consumed. Food was then returned to the tanks and reweighed after 24 hours.

6.2.3 Determining Plastic Uptake

Following the six month treatment period, the level of plastic retained by treatment group A was determined, and the presence of any environmental plastics retained in groups B and C examined. Individuals were dissected, following which the stomach was removed and preserved in 80% ethanol. The contents of the stomach were examined individually under a binocular microscope for the presence of plastics. Plastic aggregations were then weighed to 5 decimal places as outlined in Chapter Two to determine the level of contamination.

6.2.4 Hepatic Index and Plastic Consumption

Impacts of plastic ingestion on energy stores were examined by measuring the mass of the hepatopancreas. The hepatosomatic index (HSI) of each individual was determined by calculating the wet mass of the digestive gland as a percentage of total body mass (Mayrand and Dutil, 2008).

6.2.5 Total Blood Protein

To identify acute impacts of plastic ingestion, total blood protein and the concentration of haemocyanin were used as a measure of body condition. Haemolymph samples were taken from the pericardium using disposable syringes fitted with 22 gauge needles. Total blood protein was determined by the Bradford method (Bradford, 1976), using coomassie dye, which binds with protein under acidic conditions caused by the reagent, resulting in a spectral shift from red to blue.

10µl of haemolymph was diluted with 990µl of deionised water. 950µl of coomassie blue was added to 50µl of the diluted sample and the absorbance of the resulting solution was determined at 562nm using a spectrophotometer, calibrated using standardised solutions of bovine serum albumen (BSA) (Hagerman, 1983).

6.2.6 Copper Concentration

Copper concentration in the hepatopancreas was determined using atomic absorption spectrometry (AAS). Hepatopancreas samples were freeze dried over five days. Samples were then pre-digested. 100mg of dry tissue was mixed with 8ml of nitric acid. Samples were placed in a digester at 95˚C for a period of 2 hours, and then allowed to cool for a minimum of 10 minutes, following which 3ml of hydrogen peroxide were added. Samples were then left for a minimum of 8 hours, and samples made up to 10 ml with distilled water.

Samples were then analysed using atomic absorption spectrometry (AA Analyst400, Perkin Elmer Ltd, Cambridge, UK). Results were compared to standards of copper nitrate (Sigma Aldrich) diluted to concentrations of 15, 10, 5, 2.5 and 1.25 ppm and a distilled water blank.

6.2.7 Statistical Analysis

Statistical analysis was carried out using minitab15 and R statistical software package. Differences in food consumption between groups A and B were examined using a Mann-Whitney U analysis at each month. Comparisons of haemolymph protein, hepatopancreas copper, HSI, hepatopancreas water content, variation in body mass and the level of plastic between the three treatment groups were conducted using a Kruskall-Wallis test. In the event of a significant result, the relationship was explored using post hoc Mann-Whitney tests to determine the group responsible for the response.

6.3 Results

6.3.1 Survivorship and Plastic Uptake

Mortality varied between treatments groups, with the starved condition (Group C) being the least hardy (58.3% mortality), followed by plastic fed langoustine (Group A) (41.6% mortality), then fed individuals (Group B) (66.8%). The higher than expected rate of mortality is believed to be due to a complication with water flows during month four; however, it is noted that the resilience of plastic fed individuals falls between that of the starved and fed treatments.

Analysis of the plastic retained in plastic fed individuals revealed weights of between 0.00041 – 0.00349 g, and an average of 0.0015 g. One of the unfed individuals held a single pink fibre, obviously differing from the blue polypropylene used to seed individuals in the plastic fed condition. There was clear significant difference in contamination between the 3 groups at month 8 ($H = 16.77$, $df = 2$, $P < 0.001$). Unfed and plastic fed individuals indicated clear differences in carapace to weight ratio between 0 and 8 months, whilst there was no significant difference observed in fed individuals.

6.3.2 Feeding Rate

Mann Whitney analysis was used to analyse the difference in feeding rates. At 0 Months no significant difference was found between plastic fed (Group A) and fed (Group B) *N. norvegicus* (W = 156.0, P < 0.7506). Analysis of the difference in feeding rate of the fed individuals showed no significant difference between month 0 and 8 (W = 119.0, P < 0.6160), similarly, there was no significant difference between start and end feeding rates in plastic fed individuals (W = 128.5, P < 0.4988).

After eight months a difference could be observed between the 2 treatments, although this was only significant to 80% confidence (W= 44.0, P < 0.1824). This change can be observed as a steady decline in feeding over the experimental period (Figure 6.1).

Table 6.1 Average plastic recovered from each treatment group

	Plastic Recovered (g)		
	Unfed	Plastic Fed	Fed
Average	0.00000002	0.00148571	0
SE	0.00000002	0.00053914	0

6.3.3 Indices of Body Condition

Over the eight months, unfed individuals were seen to have a reduction in weight of 7.27%, and plastic fed individuals showed a weight reduction of 4.52%, a loss of 0.0303 and 0.0189 % per day. Conversely, fed individuals displayed an increase of 19.0%, equation to a gain of 0.0795% per day (Figure 6.2).

As previously indicated the carapace length to weight ratio of unfed and plastic fed individuals was seen to differ to during the experimental period. Unsurprisingly, there is also a significant difference in the percentage change in body mass between the three treatment groups ($H = 13.78$, $df = 2$, $P < 0.001$). Whilst both unfed and plastic fed individuals showed decreased body mass, individuals containing plastic were actually seen to exhibit the largest reduction in weight.

After eight months, blood protein was seen to vary significantly between groups ($H = 4.96$, $df = 2$, $P < 0.084$) (Figure 6.3). Again fed individuals had the highest protein levels, followed by plastic fed, then unfed individuals. The significant response appears to be caused by difference between the fed and un-fed controls, however, Mann-Whitney analysis also revealed weaker differences between the plastic-fed group and the two controls (A/B: $W = 24.0$ $P < 0.1939$, A/C: $W = 46.0$ $P < 0.2716$, B/C: $W = 21.0$ $P < 0.0481$).

Significant variation between groups was also observed in relation to hepatopancreas copper levels ($H = 7.96$, $df = 2$, $P < 0.019$) (Figure 6.4), however, this was driven by extraordinarily high levels in two plastic containing individuals. Mann-Whitney analysis revealed significant differences between plastic fed individuals (Group A) and both controls (Groups B & C) (A/C: $W = 42.0$ $P < 0.0128$, A/B: $W = 20.0$ $P < 0.0513$). It is unclear whether these high levels are anomalous, or the result of increased absorption from other sources. When these potentially anomalous results were excluded, the relationship was only significant to 95% ($H = 6.57$, $df = 2$, $P < 0.037$). SE of copper concentrations was found to be highest in unfed individuals, although the mean concentration was still higher than that observed in fed individuals.

Hepatosomatic Index was seen to vary significantly between the 3 groups ($H = 10.98$, $df = 2$, $P < 0.004$) (Figure 6.5). Post hoc Mann-Whitney testing revealed that this was driven by differences between individuals in the fed (Group B) and un-fed (Group C) controls (B/C: $W = 76.0$, $P < 0.0043$), and plastic fed treatment (Group A) and un-fed control (Group C) (A/C: $W= 86.0$, $P < 0.0128$). The difference between the fed and plastic fed individuals was only 50% significant. Similarly, there was variation observed between treatment and the water

content of the hepatopancreas ($H = 12.70$, $df = 2$, $P < 0.002$) (Figure 6.6). Post hoc Mann-Whitney testing revealed 95% significant differences between all groups. For both of these indexes the average response of plastic fed individuals was observed to fall between those of the starved and fed conditions.

6.4 Discussion

The results presented above are the first to indicate an impact of microplastic contamination on crustaceans, and represent the first long term contamination study in invertebrates. Whilst the study is preliminary, and uses small sample sizes, the data indicates a number of potential impacts of microplastic in *N. norvegicus* nutritional state.

N. norvegicus were seen to readily take up plastic in the aquarium. Uptake in the plastic fed condition ranged from 0.00041 to 0.00349g, averaging 0.0015g. This was over three times the average found in the Clyde, which was approximately 0.00044g on average, and far higher than that in the North Sea and the North Minch. It might then be concluded that *N. norvegicus* in the Clyde are exposed to far fewer than the 20 fibres per month added in this experiment.

6.4.1 Feeding Rate

Over the course of the study period plastic fed individuals were seen to consume less food per gram of body weight than the fed control condition. While the difference at eight months was not seen to be significantly different, there were differences observed at four and six months – with clear separation between standard error bars when displayed graphically. It may be that individuals moulting at approximately six months were relieved of their plastic loads, resulting in increased space in the gut for food.

It appears that false satiation reduced the rate of food consumption as well as the overall weight. This may result in increased opportunities for food theft by

conspecifics. In high density areas, there is a reduction in both growth rate (Tuck et al., 1997), and nutritional state (Parslow-Williams et al., 2002). This is believed to be the result of competition between conspecifics (Bailey and Chapman, 1983).

6.4.2 Metabolic Depression in Plastic Fed Individuals

The indexes related to metabolic depression both returned significant results. Haemolymph protein was seen to vary significantly between groups, with unfed individuals exhibiting the lowest levels of protein and fed individuals exhibiting the highest. This change is to be expected in animals under metabolic stress, as reduction in the metabolic rate is known to be reflected in lower levels of haemoglobin and other haemolymph proteins. The change in haemolymph protein observed in the plastic fed individuals is not as marked as that in the starved condition. It is clear that there is reduced nutrient uptake in *N. norvegicus* contaminated with plastic; however, the effect is not sufficient to prevent all nutrient uptake.

The breakdown of the main haemolymph protein, haemoglobin, results in the release of 2 copper atoms. The removal of these atoms from the haemolymph results in build-up in the hepatopancreas. Identification of potential indexes of starvation in *N. norvegicus* carried out by Watts et al. (2014) revealed that copper levels above 350.19 µg g-1 were indicative of starvation.

In the results presented, copper in the hepatopancreas was seen to be higher in both the unfed control and plastic treatment groups. This was driven by high levels of copper in plastic fed individuals. There is some uncertainty as to the high levels of copper observed in a number of unfed and plastic fed individuals, the concentrations of which far exceed those reported in Watts et al. (2014). It may be that there was an external source that influenced these results. However, all tanks were fed by the same recirculating water system and the subjects equally at risk of any waterborne pollutants.

In the plastic fed group the observed reduction in haemolymph protein and rise in hepatopancreas copper can be assumed to be caused by reduction in nutrient availability as a result of plastic contamination.

6.4.3 Reduction in Energy Stores in Plastic Fed *N. norvegicus*

Metabolic depression is only effective for limited periods, if insufficient to curb energy demands an individual must utilise its energy stores, firstly glycogen, then lipids. In *N. norvegicus*, this has been seen to result in reduction of lipid in both the hepatopancreas and tail (Barden, 1994). This reduction in lipid reserves has a range of effects to the morphology of the individual.

Studies of hepatopancreas histology in *Palaemon serratus* indicated that starvation is related to shrinking in the endoplasmic reticulum of lipid storage cells and enlargement of the mitochondria. These changes in the composition of lipid storing R-cells could be observed after only 56 hours starvation (Papathanassiou and King, 1984). Preferential catabolism of non-polar lipids has previously been observed in a range of species, for example, white shrimp, *Litopenaeus vannamei*. This is believed to be beneficial in avoiding utilization of polar lipids found in cell membranes (Sánchez-Paz et al., 2007).

In *N. norvegicus* in the wild, utilisation of energy reserves will vary between individuals dependent on factors such as activity and moult; in the lab, reductions in lipid levels have been observed from 4 months starvation (Watts et al., 2014a; Watts, 2012). As indicated above, both HSI and water content of the hepatopancreas are used as indications of depleted energy stores. The analysis of potential indicators of nutritional status in males carried out by Watts et al. (2014) revealed that HSI below 3.44% and HPW above 68.64% were indicative of nutritional stress. In a study of the nutritional value of pelleted and natural food sources carried out by Mente (2010), the starved control group exhibited a reduction in lipid concentration of 12.16% in over 8 months. If the combination

of lipid and water in the hepatopancreas equates to 80% as indicated by Watts (2014), the percentage HPW in these individuals would be approximately 67.84%.

In this study, both HSI and HPW were seen to vary significantly between the three groups, with unfed individuals exhibiting the smallest HSI and the highest HPW, and the fed control exhibiting the highest HSI and smallest HPW. The observed changes in both indexes indicate a degree of utilisation of energy stores in both unfed and plastic fed individuals.

The results presented indicate a reduced nutritional state in the plastic fed group, albeit less than that of the starved condition; however, the level of plastic observed in the contaminated group is higher than that observed in the langoustine recovered from the CSA. This suggests that the impact of plastic ingestion on wild langoustine will be less severe.

6.4.4 Long Term Impacts of Plastic Ingestion by *N. norvegicus*

Long term dependence on energy reserves such as lipids is known to result in decreased body mass. In her 8 month study of the effectiveness of natural and pelleted diets in *N. norvegicus*, Mente (2010) saw a decrease in average body mass in starved individuals of 0.02% per day. The increase in body mass of the fed conditions was dependent on the quality of the diet, varying between 0.06% and 0.08% per day.

In the present study, fed individuals gained an average of19.0% body mass over the 8 months – which equated to 0.0795% daily. The body mass of unfed individuals reduced by 7.27% over 8 months, on average 0.0303% per day, this was greater than that observed by Mente (2010). Plastic fed individuals fell between that of the two controls, losing an average of 4.52% of original body mass, equating to 0.0189% per day. This loss of body mass is assumed to be the result of decreased uptake of nutrients, leading to long term utilisation of stored energy.

The extent of weight lost in the plastic group was surprising, as they were still observed to be feeding, albeit at a reduced rate. It may be that the plastic in

the gut is further reducing nutrient uptake, this would reduce the effective nutritional value of any food consumed. This may be the result of damage to the gut wall. Although relatively unstudied in invertebrates, ingested HDPE has been seen to cause an inflammatory response in the tissues of *Mytilus edulis* (von Moos et al., 2012), a similar effect in the tissues of the gut would reduce effective nutrient transport.

In the wild, there may be high variation in these impacts as a result of individual differences. Annual variation in the effect of microplastic uptake may vary with moult stage. Before moult, feeding rate is decreased during the removal of calcium from masticatory structures, and does not return to normal until these structures are hard enough to cope with feeding (Phlippen et al., 2000). As a result, individuals already subject to nutritional stress due to high plastic loads would lack the necessary reserves to undergo this fasting period. There may also be variation in impact between males and females. Ovigerous females only moult once a year, as opposed to twice in males and immature individuals. Brooding females then have fewer opportunities to egest their plastic load. The period immediately before moult may be crucial to plastic contaminated individuals.

Reduction in body weight has a number of impacts on biological processes. Brooding females also have a reduced feeding rate as a result of increased residence in their burrows (Farmer, 1975). In a number of crustacean species including *N. norvegicus*, body size in females is strongly linked to fecundity (Abellô et al., 1982; Beyers and Goosen, 1987; Hines, 1991; Lizárraga-Cubedo et al., 2003). Reduction in body mass related to plastic contamination may result in decreased egg production. Relationships have previously been observed between lipid levels and larvae growth, ovarian maturation, spawning capacity in *Penaeus japonicus* (Kanazawa et al., 1979). Similarly, in his 1990 review, Harrison analysed the available data on the use of carbohydrates, lipids and proteins in the various stages of egg formation, finding lipids to be of high importance in both oogenesis (egg formation) and vitallogenesis (yolk formation).

The ability of langoustine to egest plastic would reduce the risk of mortality related to microplastic ingestion; however, it may reduce an individual's ability

to adapt to other stressors. The mortality observed across all groups during the study is higher than that observed in previous studies. This is thought to be the result of disrupted water flow in month 4. However, the resilience of individuals to this disruption varied between groups, with starved individuals demonstrating by far the highest mortality rate, and the fed control individuals the lowest. A possible impact of the reduction in water flow is decrease oxygen availability and an associated decreased in the ability to catabolise lipids, which require more than twice the oxygen per gram to break down (Schmidt-Nielsen, 1997).

Blood protein in wild caught *Crangon vulgaris* was seen to vary with the stages of the moult cycle (Djangmah, 1969), a similar response, between four- and five-fold, was observed in *Carcinus maenas* at moult (Busselen, 1970). Lipid content of the hepatopancreas has also seen to vary with the moult cycle in a number of crustaceans (Chang, 1995).

6.4.5 Applicability to Other Species

N. norvegicus are an ideal species in which to study long term plastic exposure, as they are known to retain plastic throughout their moult cycle. As a result, the animals sampled here displayed a clear reduction in physical condition compared with that of the fed control. However, the results of this experiment may not be applicable to other invertebrate groups, particularly those with different gastric structures, in which plastic is more readily passed. There is still little information on the frequency of uptake and length of microplastic retention of in many marine invertebrates. In many species long term contamination may not be of high concern.

Examination of plastic retention time in other species, particularly crustaceans, may reveal those at greatest risk of nutritional impacts; however we must be careful in extending these results to other species. Crustaceans are adapted to cope with periods of starvation related to low food availability and the moult cycle. Within the crustacea there are highly varied patterns and rates of nutrient uptake and utilization of energy stores, for example utilisation of lipids by starved *Penaeus esculentus* is seen to occur after as little as seven days (Barclay

et al., 1983), whereas decreases in lipid concentration in *N. norvegicus* began at approximately four months (Watts et al., 2014a).

Despite greater ability to egest plastic, impacts of microplastic contamination have been observed in short term studies of other invertebrate species; for example, in *Arenicola marina*, decreases have been observed in both feeding rate and body weight in relation (Besseling et al., 2012). Similarly, filtering in *Mytilus edulis* exposed to nano-polystyrene was seen to result in decreased filtering in activity (Wegner et al., 2012).

Whilst there are no available studies of comparable length, examination of the impact of plastic consumption on energy stores has been carried out in organisms with a shorter lifespan; *A. marina* kept in UPVC contaminated sediments displayed uptake of energy reserves of up to 50% over four weeks (Wright et al., 2013).

6.4.6 Further Study

As a preliminary study, the results presented highlight the need for greater research into the impacts of long term microplastic ingestion on invertebrates, particularly those susceptible to other anthropogenic stressors. Other commercial crustacean species are particularly at risk, and further information is required as to the impact of plastic on fecundity.

As indicated in the previous chapter, the gut volume of langoustine was seen to vary with carapace length. As a result of the increased food capacity and lower levels of plastic observed in larger individual, the impact of plastic ingestion may be reduced.

There may also be differing impacts in the effects observed with varying microplastic size. The effects of ingestion of microspheres ranging from 0.05, 0.5 and 6 µm diameter was analysed in the copepod *Tigriopus japonicas*. It was found that beads at 6um did not greatly impact survivorship, whilst the smaller 0.05 um sample caused significant decreases in survivorship of both adults and

nauplii. The intermediate size class indicated an impact only at higher concentrations (Lee et al., 2013).

6.5 Summary

In this preliminary study, plastic fed *N. norvegicus* exhibited a lower feeding rate over a number of months. This was higher than that previously observed from wild caught individuals.

There was an obvious decrease in the nutritional state in the plastic fed group. This was observable as a fall in metabolic rate, demonstrated by reduced levels of protein in the haemolymph and increased copper in the hepatopancreas. There was also a decrease in the indexes of stored energy. The proportion of water in the hepatopancreas and the hepatosomatic index were both seen to change with plastic consumption, indicating catabolism of lipid reserves.

The body mass of plastic fed *N. norvegicus* was seen to decrease over the experimental period. At this point it is not possible to isolate the impacts of reduced feeding rate caused by false satiation from potential nutrient dilution caused by damage to the gut. Future experiments exposing plastic fed langoustine and clean fed langoustine to a reduced diet, thus equalising the amount of food ingested across the groups, may expose potential reduction in nutrient uptake caused by microplastic contamination.

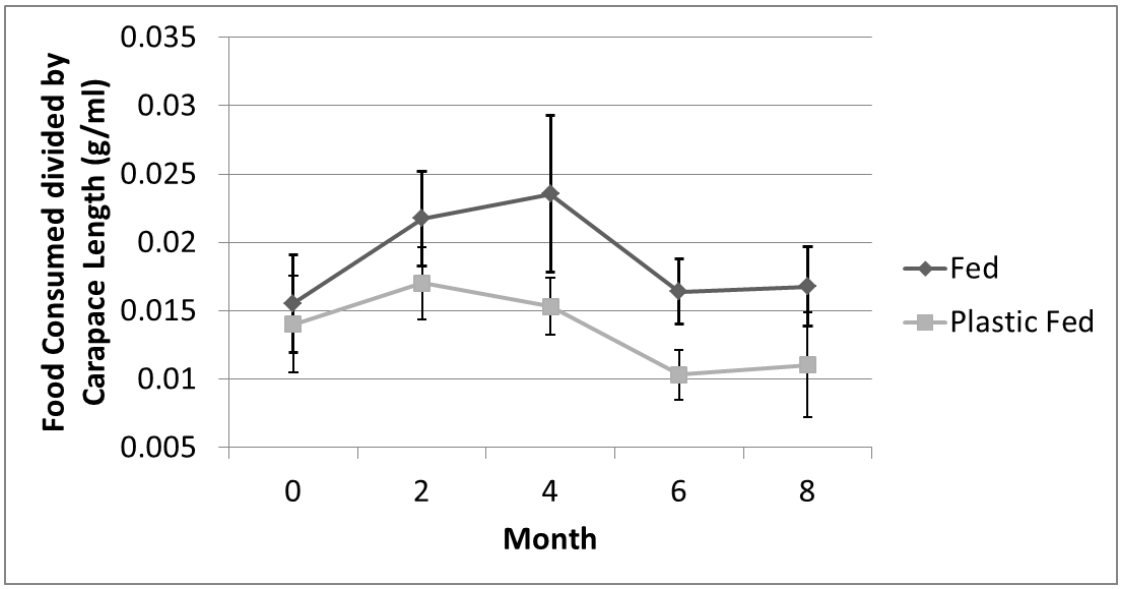


Figure 6.1 Average food consumption (g) divided by carapace length mm

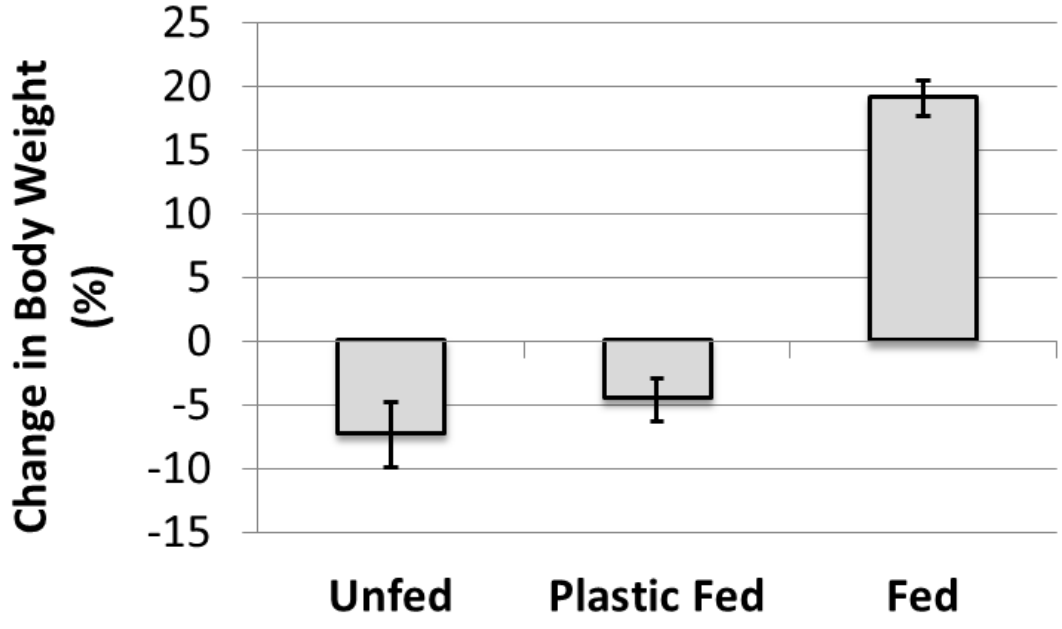


Figure 6.2 Percentage Change in Body Weight after Eight Months

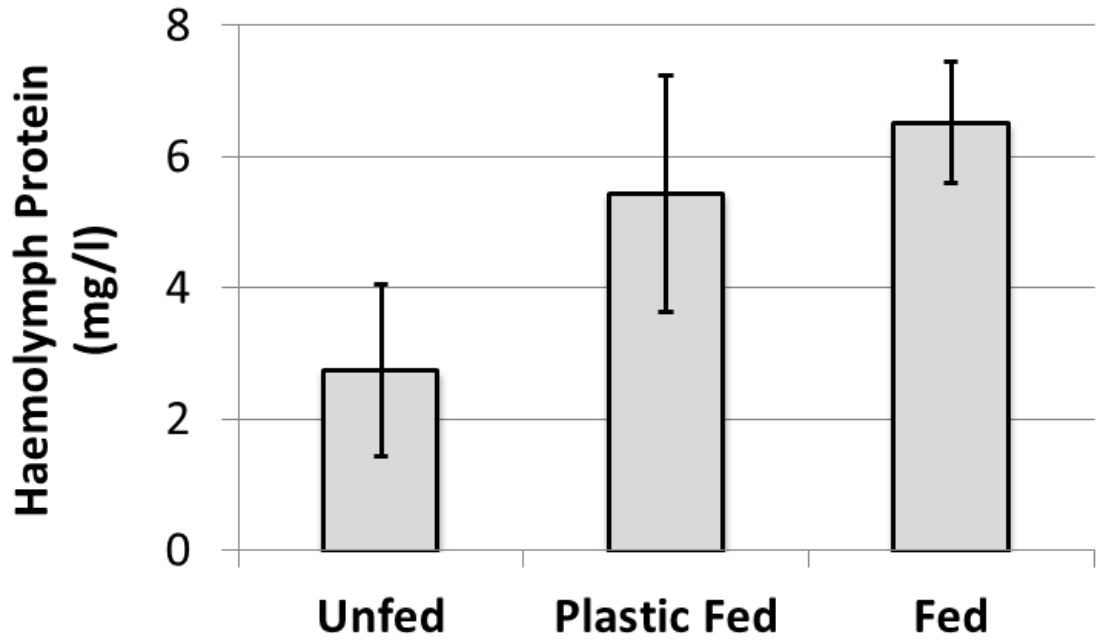


Figure 6.3 Variation in Haemolymph Protein after Eight Months

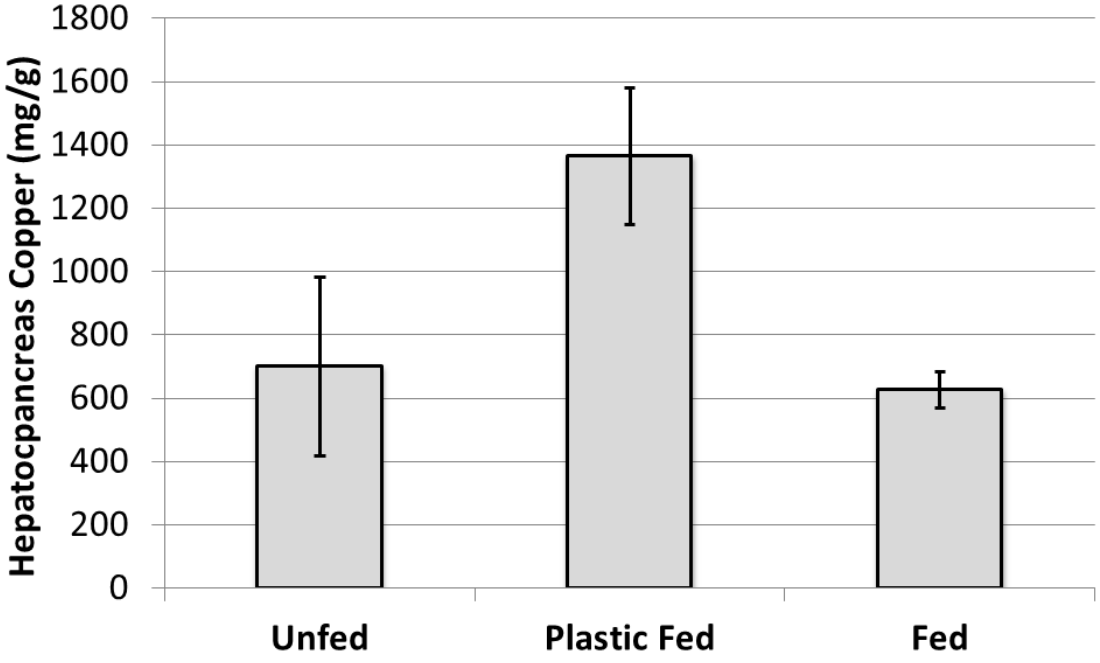


Figure 6.4 Variation in Hepatopancreas Copper Observed after Eight Months

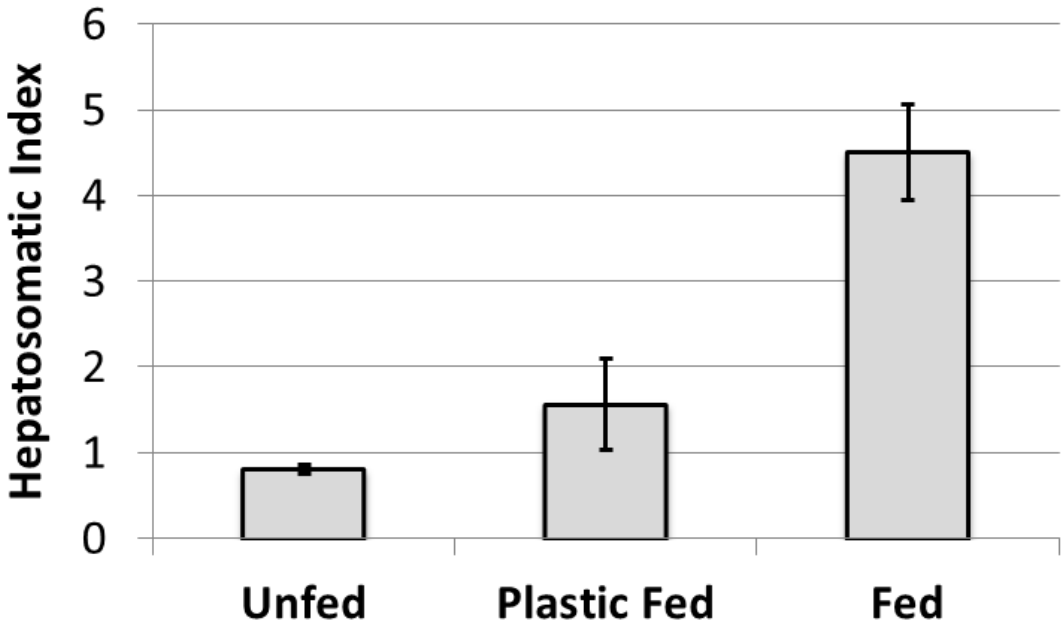


Figure 6.5 Hepatosomatic Index of Each Group, Observed after Eight Months

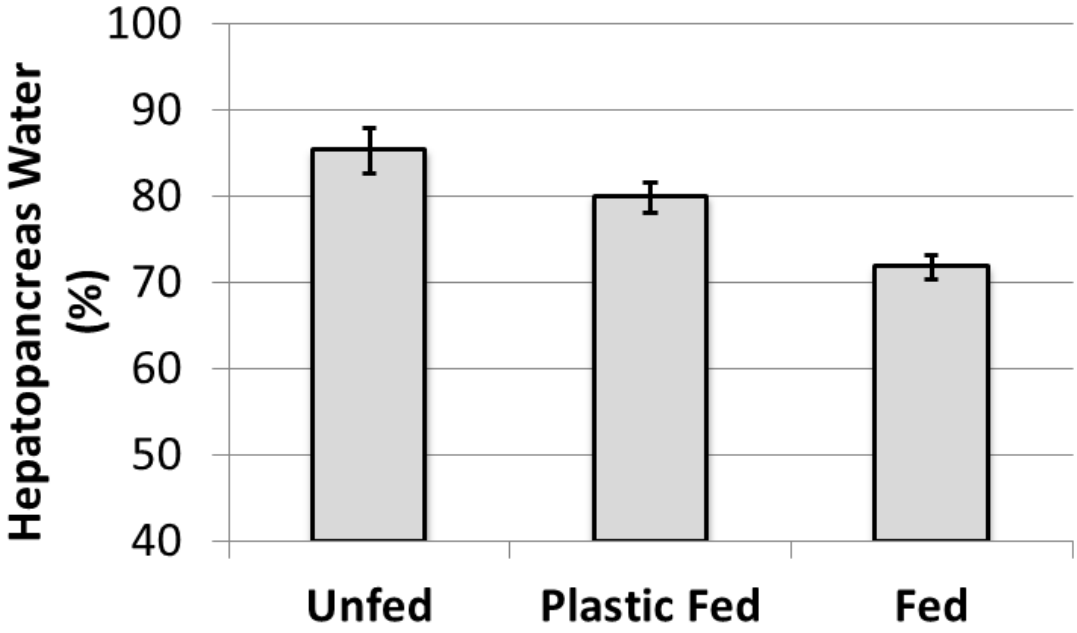


Figure 6.6 Variation in Hepatopancreas Water between Groups, Observed at Eight Months

Chapter 7 General Discussion

7.1 Summary of Results

Although there have been many developments in the study of marine microplastic in the past decade, the distribution and quantity of data is often limited to monitoring levels in either sediment, the water column, or biota. In order to expose the dynamics of microplastic transport within an ecosystem, we used the Clyde Sea Area (CSA) as a model, to gain a holistic view of microplastic aggregation and distribution. The results of Chapter Two are the first to identify variation in microplastic aggregation in an organism in relation to location and proximity to pollution sources; Chapters Five and Six identify previously unknown causes of plastic aggregation and removal, and the impacts of plastic retention on *N. norvegicus*. Chapter Four aimed to illuminate the formation of microplastics within the CSA, by quantifying preliminary rates of degradation of commonly used polymer ropes. Spatial and temporal fluctuation in the level of microplastic debris in the sediment and water column was examined in chapter six.

7.1.1 The Formation and Distribution of Microplastic in the CSA

Many studies have identified proximity to sources of plastic pollution as a cause of elevated microplastic concentrations (Claessens et al., 2011; Reddy et al., 2006). Analysis of samples collected from four sites in the CSA revealed microplastic concentrations in the water column and sediment which correspond to those observed in other highly populated areas (Claessens et al., 2011; Ng and Obbard, 2006). The low level of water exchange with the Irish Sea indicates that there will be limited influxes of plastic from the North Channel (Davies and Hall, 2000; Dooley, 1979).Therefore, the recovered microplastics are believed to originate in the Clyde catchment; their sources are thought to be a mix of plastics from the Clyde catchment released with the washing of clothes and passage of pre-production pellets and scrubs, as well as weathering of plastics already in the marine environment.

The riverine water inputs which introduce plastics into the CSA also form a low salinity surface layer (Poodle, 1986). Lower salinity leads to a decreased water density, which would result in reduced buoyancy of the polymer relative to that observed in more saline conditions. As a result, the initial rate of polymer sinking would be increased, and more plastics are expected to be deposited higher up the CSA.

Analysis of the distribution of plastic was carried out over seven months, the longest repeated site monitoring program to date. Whilst the results displayed spatial variability both on the small scale and across the CSA, there was no eveidence for increased deposition in sites higher up the CSA. There was high variability observed between months, and the impact of storm events had a great effect on the level of microplastic recovered.

Previously, little was known about the formation of microplastics in the marine environment, and much of the available data is the result of exposure of polymer films. In this thesis we utilized ropes commonly used in maritime activities around the CSA. Analysis of rope degradation in shallow sub-tidal waters indicates a rate of input of up to 0.422 g per meter per month. This rate may be expected to increase with the surface area of rope available to both abiotic and biotic factors.

The colonisation of microplastics observed over only 4 months in low light indicates that floating debris in the CSA may be a vector for transporting species to other areas (Lewis et al., 2005). There was no non-native species recorded on any other of the ropes over the 12 month exposure period; however, a number of non-native sessile organisms such as the leathery sea squirt, *Styela clava* (Dupont et al., 2010), the carpet sea squirt, *Didemnum vexillum* (Murphy, 2010), have been identified in nearby harbours. Plastic debris leaving the CSA may result of transport of these organisms to other areas.

7.1.2 Microplastic in *Nephrops norvegicus* in the CSA

Prior to the commencement of this study, microplastic aggregations had been observed in 83% of *N. norvegicus* recovered in the CSA (Murray and Cowie, 2011); however the environmental and biological factors responsible for this aggregation were unknown. In this thesis it was found that the *N. norvegicus* recovered from the CSA displayed significantly higher occurrence and aggregations of gut microplastic than those collected from the North Sea and North Minch. This variation is believed to be the result of the high number of local sources of contamination in the CSA.

Many of the individuals sampled from the CSA contained large aggregations that most have accumulated over a large period. The relationship between weight of plastic and moult stage in wild caught *N. norvegicus* from the CSA indicates that microplastic is aggregated throughout the intermoult period. In laboratory experiments, *N. norvegicus* fed contaminated squid rations were observed to aggregate microplastic within the stomach. This is believed to be the result of the microplastics, particularly fibres, being unable to pass through the gastric mill. Larger individuals appear to be less susceptible to plastic contamination, as the gaps between the teeth of the gastric mill are much larger, allowing a greater proportion of fragments and fibres to pass through.

As a result of their increased gut complexity over other invertebrate species, *N. norvegicus* appear to be at greater risk of long term plastic contamination and associated biological impacts. However, the moult cycle allows individuals to egest microplastic aggregations (Figure 7.1), as the stomach lining is expelled at ecdysis. In the laboratory, individuals fed with microplastic showed large aggregations in the shed gut lining, demonstrating that *N. norvegicus* are capable of reducing their plastic load. The dependence on moult to egest accumulated microplastic highlights a greater threat to ovigerous females, in which moult interval is increased from 6 to 12 months.

In the first long term study of microplastic contamination in any invertebrate, a definite reduction in body condition was observed (Table 7.1). Individuals fed microplastic contaminated squid exhibited reduced metabolism and lower lipid reserves. This resulted in reduced overall body mass. While the levels of plastic exhibited in laboratory animals were higher than most individuals in the CSA, it was comparable with that of small female *N. norvegicus*, whose plastic loads were highest overall. It is therefore probable, that small females would exhibit reductions in body condition similar to those observed here.

7.1.3 Cycles of Plastic in the CSA

While a number of studies have been carried out on the distribution of environmental microplastic (Browne et al., 2011; Claessens et al., 2011; Galgani and Andral, 1998; Reddy et al., 2006), there is minimal information on variation in microplastic distribution over time. Throughout the presented results, variation has been observed in the levels of faunal and environmental microplastic aggregation. In environmental microplastics, redistribution will be caused by turbation of the sediment by storms (Lattin et al., 2004) and trawls (Churchill, 1989; Pilskaln et al., 1998). The rate and location of settlement will be determined by the environmental conditions immediately following these events (Ballent et al., 2012; Barnes et al., 2009; Williams and Tudor, 2001). The lack of observable relationship between sediment depth and level of microplastics suggests a homogenisation of the surface layers, which may also be a consequence of repeated trawling by commercial vessels.

At this point there is little information on the transfer of plastics through the food chain. While there was a similarity in the plastics recovered from *N. norvegicus* and those from sediment, it is unclear as to whether this is taken up directly or as a result of trophic links through benthic dwelling organisms. Due to the long residence time of plastic in the gut, there were no observable links between particular food items and ingested microplastics. At present, only a handful of studies have identified the trophic transfer of plastic, many of which took place under laboratory conditions and used levels of microplastics much higher than those found in the environment.

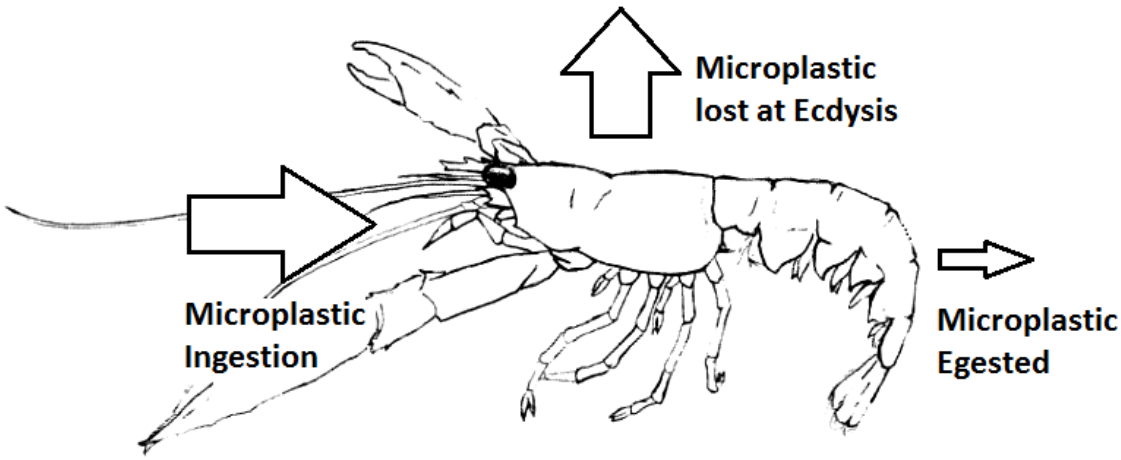


Figure 7.1 The Uptake and Egestion of Microplastic in *N. norvegicus*

Table 7.1 Response of *N. norvegicus* Indices of Nutritional Health Following Eight Months Exposure to Microplastic

Index of Nutritional State	Response	Meaning
Haemolymph Protein	↓	
Hepatopancreas Copper	↑	Copper released from haemocyanin stored in hepatopancreas
Hepatopancreas Water	↓	Catabolism of lipid produces tissue water
Hepatosomatic Index	↓	Decrease in lipid reserves

Transfer of microplastic through the food chain has yet to be observed outside the laboratory; however, if trophic transfer were to be observed in any animal in the CSA, *N. norvegicus* would be the most likely. As unselective scavengers, *N. norvegicus* consume a range of species with varying feeding modes, including filterers and deposit feeders (Cristo and Cartes, 1998). This increases the number of links between *N. norvegicus* and potential plastic inputs, increasing the risk over that of species that rely on specific prey. *N. norvegicus* also eat conspecifics (Cristo and Cartes, 1998), individuals consuming other plastic contained individuals may immediately gain a large plastic load.

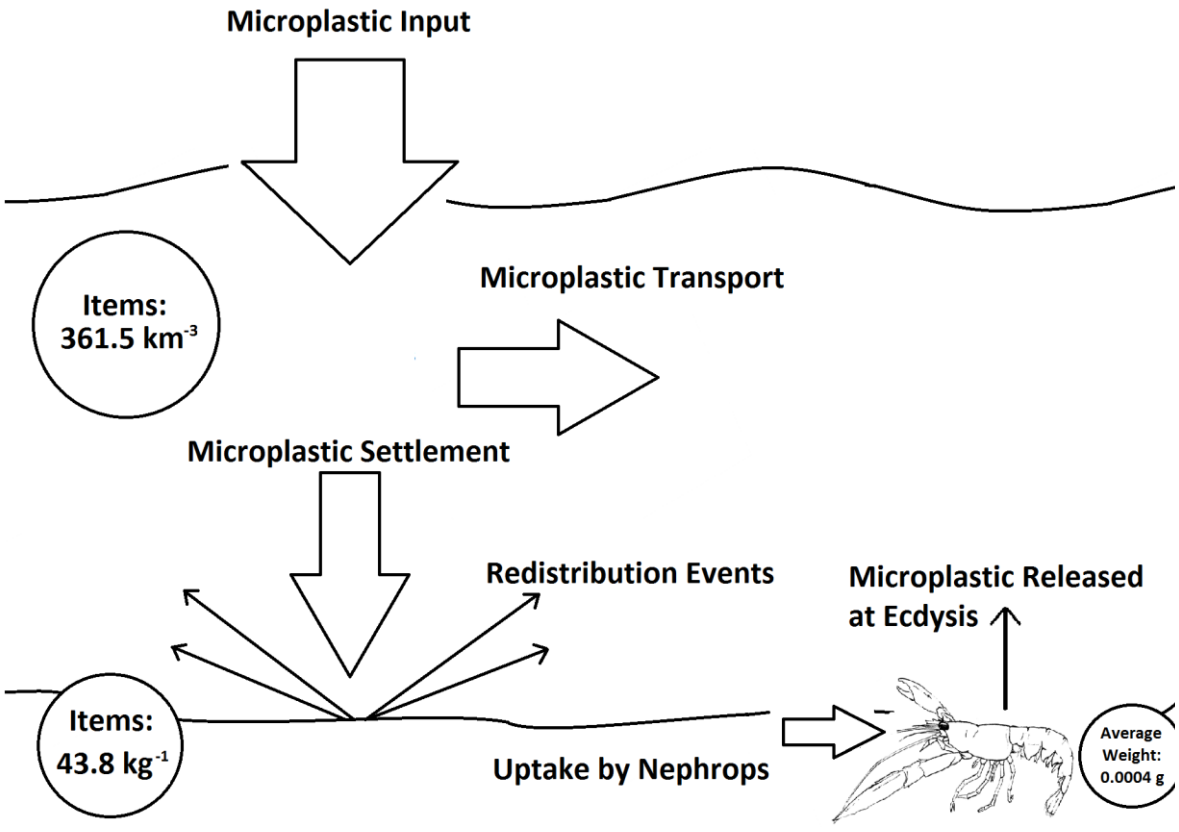


Figure 7.2 The Distribution and Observed Cycles of Microplastic in the CSA

7.2 Beyond the Clyde

Outwith the CSA there is an ever increasing amount of information on the level and fate of plastic pollution. However, the distribution of this data is generally dependent on proximity to the particular research group. In remote areas the volume of data become patchy, usually the result of obvious and abnormally high aggregations of debris. With the exception of two recent studies on zooplankton (Frias et al., 2014), and *Mytilus edulis* (Mathalon and Hill, 2014), there is limited information on how the level of microplastic contamination in the environment relates to that in marine fauna. As a result, the ability to draw comparisons between regions is greatly reduced.

Studies comparing levels of both environmental and faunal microplastic not only identify local levels of contamination but enable researchers in other areas to extrapolate the threat to the biota based solely on environmental contamination (and vice versa). The comparison between yearly fluctuation in environmental microplastics and those recovered from *N. norvegicus* may be used to identify other areas at which *N. norvegicus* and other crustaceans are at risk. The concentrations of microplastic observed in both the water column and sediments of the CSA were similar to those found in other estuarine regions. This suggests that crustaceans living in those environments are also at risk of microplastic aggregation. Global increases in marine microplastic debris may result it increased uptake of microplastic by *N. norvegicus* in previously low impact locations. The result of which will be comparable to those currently observed in the CSA.

7.3 Limitations of the Work

The gut content analysis performed in this thesis was not able to identify the rate of plastic uptake in *N. norvegicus*. While transfer of fibres seeded in squid mantle was observed in the laboratory, there is currently no evidence that

uptake of plastic occurs through the food chain. While there was a reduction in the nutritional state of plastic fed individuals in the laboratory experiments presented, the average weight of plastic observed was greater than that recorded in wild caught individuals. Thus actual impact of microplastic ingestion by *N. norvegicus* in the wild may be less than that observed in Chapter Six, and is expected to vary in relation to microplastic load.

7.4 Future Work

7.4.1 Microplastic Monitoring.

With the introduction of the Marine Strategy Framework Directive there has been increasing discussion regarding the most suitable method of sampling for microplastics in both water and sediment (Claessens et al., 2013; Galgani and Andral, 1998; Hidalgo-Ruz et al., 2012). Suggested water column sampling techniques vary between bongo nets or manta trawls, benthic sediment has been collected by cores and grabs, and beaches have been surveyed using everything from box cores to spoons (Hidalgo-Ruz et al., 2012). Sampling method aside, the monthly variation in microplastic recovered from both the sediment and water column in the CSA indicates that yearly sampling may be insufficient to capture the true level of plastic contamination.

Variation between sites and even samples at a single location suggest that the level of spatial variation is too high to capture in a handful of samples. As a result, the use of indicator species may be more appropriate. *N. norvegicus* are prime indicator species for microplastic debris, appearing to reflect the density and composition of plastic pollution. Their tendency to aggregate microplastic over a number of months would result in a representative profile of that available in the sediments.

Laboratory exposure may be used as a route for establishing the uptake rates of different species at varying concentrations, allowing comparability between studies in different ecotypes. *Mytilus* sampled from the upper CSA have also

been seen to contain microplastics taken up from the marine environment. *Mytilus* have previously been used to monitor contaminants such as heavy metals in benthic habitats (Goldberg et al., 1978); it would not be a stretch to develop a similar protocol for microplastics.

7.4.2 Determining the Impacts of Ingestion

There are increasing numbers of short term experiments on the impacts of plastics and their contaminants on invertebrates (Besseling et al., 2012; Wright et al., 2013). However, few of these are standardised by ecologically sound ingestion rates and retention times. In order to accurately assess the potential impacts of microplastic contamination in invertebrates it is essential that we identify and prioritise those species that may be at greatest risk.

The transfer of contaminants from plastics to organisms has been the focus of a number of recent papers (Gouin et al., 2011; Mato et al., 2001; Teuten et al., 2007; Teuten et al., 2009); however, most of these studies use concentrations of plastics and contaminants far above those recorded in the environment. The most commonly used plastics in these experiments are microspheres; not the fragments and filaments commonly ingested by invertebrates. The microspheres have a low surface area to volume ratio, reducing the rate of contaminant exchange between the polymer structure and surrounding tissue, and many not represent the potential impacts to the organism.

Recent modelling work carried out by Koelman et al. (2014) proposes a further interesting point. At or near the source of debris many of the plastics will have levels of contaminants much *lower* than those of the surrounding environment and its inhabitants. Partitioning may actually serve to *remove* chemicals from the environment or any organism that may consume them. In the case of the CSA, newly released plastics may be absorbing hydrophobic contaminants, slowly transporting out and into the Irish Sea. In *N. norvegicus*, the long residence time may allow the removal of contaminants, allowing them to be moulted away during ecdysis.

7.4.3 Reducing the Impact of Microplastic

The last decade has seen a great increase in public awareness of plastic pollution, and more recently the increasing magnitude and threat of microplastics (Ebbesmeyer, 2009). We have come a long way from regarding plastics as merely aesthetically displeasing; however, we have yet to erase the image of plastics as throwaway items. Removal of plastic from the environment has so far been minimally successful. Fishing for plastic is unsuitable for the collection of small plastic debris, and beach cleans are reliant on the availability and willingness of volunteers.

The biggest challenge is finding a suitable replacement for plastics. Unfortunately, the great range and durability of plastics is essential for a range of applications, for example medical devices and electronics. The task of developing a product that can meet the impressive array of material properties, without the associated impacts on the environment, is a complicated one. In some cases, the solution has been to look back, to traditional materials such as cloth and glass. At the point of writing, a number of towns and cities have successfully reduced the usage for free plastic carrier bags, and San Francisco was taking the next obvious step, banning all plastic bottled water.

Worldwide, there have been increases in the use of “degradable” plastics, and these are suitable for a range of single use applications. For those items for which there is no alternative than to use plastics, it may be possible to switch the polymer. For example, using only high density polymers would decrease the ranges over which plastics disperse before sinking; limiting the impact of microplastic releases to smaller areas (Browne et al., 2010). Rapid sinking away from the photic zone would also reduce the time exposed to UV radiation, reducing the rate at which microplastics are formed (Kinmonth, 1964).

Whilst there is currently a great deal of public pressure for the reduction of plastic litter, in the short term, cessation of plastic input into the marine environment would only be observable in changes in macroplastic and primary

microplastic debris. Secondary microplastics will continue to be formed by the fragmentation of marine debris for decades to come; this would be observed as in increase in the proportion of these fragments over other forms of marine litter, as described by Browne et al. (2010). While changes in both legislation and engineering will provide the means to reduce plastic debris, the need for research into the effects of microplastics is as great as it was ten years ago.

7.5 Summary

The long residence time of plastic in the gut of *N. norvegicus*, indicates that there may be high transference of any additives to the organism (provided that the concentration of contaminants are sufficient). The large numbers of fibres observed also result in a high surface area to volume ratio, increasing transfer rate over that of fragments or nibs. Identifying the route of plastic uptake in *N. norvegicus* is imperative in determining cycles of microplastic through the food chain in the CSA. This may also indicate other species at risk of plastic ingestion through prey. The spatial and temporal variation in plastic is too great to be encapsulated in regular sampling events. Therefore, a suite of indicator species is suggested as a representative alternative.

While there are still gaps in the knowledge surrounding the movements and impact of microplastic, there is little doubt that microplastics are affecting the marine environment. Finding alternatives to plastic products is currently both difficult and costly. It is hoped that increased pressure from statutory bodies in the wake of the marine strategy framework directive, will lead to increased pressure on companies to reduce both plastic components and packaging of goods, and that those which do not will be clearly labelled, helping the consumer to improve their buying choices.

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