Assessing environmental distribution and stakeholder awareness of microplastics: A case study in Dundalk Bay

Stephen Kneel, B.Sc. (Hons)

A thesis presented to the School of Health and Science, Dundalk Institute of Technology in fulfilment of the requirements for the degree of Doctor of Philosophy

Dr. Suzanne Linnane (Principal Supervisor), Centre for Freshwater and Environmental Studies, Dundalk Institute of Technology.

Dr. Caroline Gilleran Stephens (Secondary Supervisor), Centre for Freshwater and Environmental Studies, Dundalk Institute of Technology.

Dr. Alec Rolston (Advisor), Goyder Institute for Water Research, South Australia

August, 2024

Declaration Page

We, the undersigned declare that this thesis entitled "**Assessing environmental distribution and stakeholder awareness of microplastics: A case study in Dundalk Bay"** is entirely the author's own work and has not been taken from the work of others, except as cited and acknowledged within the text.

The thesis has been prepared according to the regulations of Dundalk Institute of Technology and has not been submitted in whole or in part for an award in this or any other institution.

Author Name: Stephen Kneel

Author Signature: Stephen Kneel

Date: 14/08/24

Supervisor Name: Suzanne Linnane

Supervisor Signature: Suzanne Linnane

Date: 15/08/24

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“But you, with Pleasure own your errors past,

And make each Day a crtic on the past”

Alexander Pope

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List of Abbreviations

ALDFG	Abandoned, Lost, Discarded Fishing Gear
AREC	Association of Research Ethics Committees
CCD	Charged Coupled Device
EVOH	Ethylene vinyl alcohol
ECHA	European Chemicals Agency
EDCs	Endocrine Disrupting Chemicals
EPA	Environmental Protection Agency
EtOh	Ethanol
FAO	Food and Agriculture Organisation of the United Nations
FTIR	Fourier-transform Infrared Spectroscopy
GES	Good Environmental Status
GESAMP	Group of Experts on the Scientific Aspects of Marine Environmental Protection
Gg	Gigagram
GIT	Gastrointestinal tract
H_2O_2	Hydrogen Peroxide
HCl	Hydrochloric Acid
HDPE	High-density Polyethylene
HNO_3	Nitric Acid
IUCN	International Union for Conservtion of Nature
K_2CO_3	Potassium Carbonate
KOH	Potassium Hydroxide
LDPE	Low-density Polyethylene
MPs	Microplastics
MSFD	Marine Strategy Framework Directive
Mt	Megaton
NaCl	Sodium Chloride
NaI	Sodium Iodide
NaOH	Sodium Hydroxide

NEPs	National Enforcement Policies
NOAA	National Oceanic and Atmospheric Administration
PA	Polyamide
PAHs	Polycyclic Aromatic Hydrocarbons
PBDEs	Polybrominated Diphenyl Ethers
PBT-PTMG	Poly(butylene terephthalate)-poly(tetramethylene glycol)
PC	Polycarbonate
PCBs	Polychlorinated biphenyls
PCT	Polycyclohexylenedimethylene terephthalte
PE	Polyethylene
PE	Population Equivalents
PES	Polyethersulfone
PET	Polyethylene terephthalate
PEz	Pancreatic Enzymes
POP	Persistent Organic Pollutant
PP	Polypropylene
PPCPs	Pharmaceutical and Personal Care Products
PS	Polystyrene
PU	Polyurethane
PVC	Polyvinylchloride
RBMPs	River Basin Management Plans
ROI	Republic of Ireland
ROI	The Republic of Ireland
SAC	Special Area of Conservation
SDGs	Sustainable Development Goals
SEP	Polystyrene-poly(ethylene propylene)block-polystyrene
SOM	Soil Organic Matter
SOPs	Standard Operating Procedures
SPA	Special Protected Area
SPT	Sodium Polytungstate

TAC	Total Allowable Catch
UV	Ultraviolet
WWTP	Wastewater treatment plant
$ZnCl_2$	Zinc Chloride

Assessing environmental distribution and stakeholder awareness of microplastics: A case study in Dundalk Bay

STEPHEN KNEEL

Abstract

Microplastics have fast become a pollutant of ubiquitous nature in the environment, documented in pristine and remote regions worldwide and recently in humans. While studies on microplastics in marine environments are more established, comparatively understudied is the freshwater environment, with freshwater research generally focusing on larger rivers. Further to this, little work has been completed understanding the social aspect of microplastics despite it being an anthropogenically-caused pollutant. Additionally, the majority of studies completed on assessing microplastic presence are one dimensional in nature focusing on one environmental compartment, however, microplastics released into the environment can interact with numerous biota and travel between terrestrial, freshwater and marine systems. In light of these factors the research in this thesis therefore presents a holistic approach to microplastic pollution in Dundalk Bay and its associated freshwater inputs, while examining stakeholders in Irish fishing relationship with plastic. An important nursery for all commercial fish species in the Irish Sea, sustaining both a productive cockle and razor clam fishery and serving as a vital overwintering refuge for thousands of seabirds, the ecological and economic benefits of a healthy ecosystem here are numerous. In spite of these factors Dundalk Bay has until now been unstudied in terms of microplastics pollution and while its shallow nature with many freshwater inputs lend to a productive environment these factors may contribute to the accumulation of microplastics here and it being a hotspot for this pollutant. The results of this study indicate that microplastics are polluting the marine environment and associated freshwater environment of Dundalk Bay. Microplastics primarily fibrous in nature were documented in surface water, sediment and G. duebeni examined in freshwater rivers as well as in marine intertidal sediments and inhabitants of this shallow marine environment. Those surveyed within the Irish fishing community were aware of microplastic pollution pertaining to aquatic environments moreso than the terrestrial and noticed litter frequently and in large quantities when taking part in fishing activities but were also likely to remove it from the environment highlighting the role that fishers can have in reducing secondary microplastic pollution in more remote environments. The ubiquitous presence of microplastics in environs studied in this thesis highlight the need for mitigation with regards to this pollutant entering the environment.

Chapter 1: Literature review

1.1 General Intoduction

1.1.1 Plastics; their uses and Issues

Some of the properties that plastics are lauded for, such as temperature resistance, resilience to abrasion, and hydrophobicity allow plastics to exist in the environment many years after their purpose as a consumer good has been carried out. Today plastic types and their uses are wide-ranging with polyethylene the highest volume global plastic available with both high- and low-density variants making up over 28% of Europe's demand in 2021, and world-wide plastic production reaching 390.7 Mt (Fig. 1-1, Plastics Europe, 2022). Although many types of polymers exist, polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC) and polyurethane (PU) make up 75% of total plastic demand (Bellasi *et al*. 2020). Other common types of plastics, their uses and densities are diplsayed in table 1-1 below. However, just as plastic production has increased so too has plastic waste and pollution to the detriment of the environment. In high-income countries the percentage of total solid waste made up by plastic had increased from 1% in 1960 to over 10% in 2005 (Wagner, 2017). Furthermore, only 9% of plastic waste has ever been recycled, while 12% has been incinerated with the remaining 79% accumulating in natural ecosystems (Geyer *et al.* 2017).

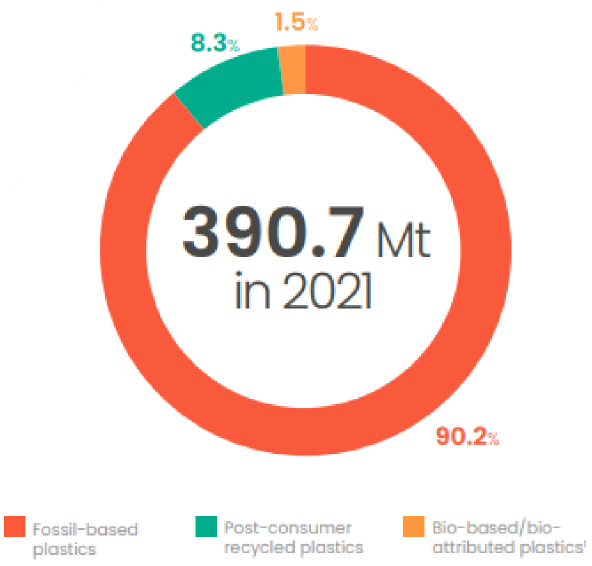


Figure 1-1: World Plastic production in 2021 (Plastics Europe, 2022).

Table 1-1: Commonly produced polymers, their associated densities and uses (Choon et al., 2021).

Polymer	Abbreviation	Min. Density (g/cm^3)	Max. Density (g/cm^3)	Main Application
Polyethylene	PE	0.91	0.97	Packaging
Polyester	PES	1.24	2.3	Textiles
Polyethylene terephthalate	PET	1.37	1.45	Packaging
Polystyrene	PS	1.01	1.04	Packaging
Expanded polystyrene	EPS	0.016	0.640	Food packaging, construction material
Ethylene vinyl acetate	EVA	0.92	0.94	Others
Alkyd	Al	1.67	2.1	Paints, fibers
Polyvinyl chloride	PVC	1.16	1.58	Building and construction
Polymethyl methacrylate	PMMA	1.17	1.2	Electronics (touch screens)
Polyamide (nylon)	PA	1.02	1.05	Automotive, textiles
Polyacrylonitrile	PAN	1.09	1.2	Textiles
Polyvinyl alcohol	PVOH	1.19	1.31	Textiles
Acrylonitrile butadiene styrene	ABS	1.06	1.08	Electronics
Polyurethane	PUR	0.03	0.1	Building and construction

1.1.2 Microplastics: An introduction and their spread

The term microplastic is a relatively new one, first coined in a study titled "Lost at Sea: Where is All the Plastic?" on the presence of microscopic plastic pieces in marine sediment and waters in the UK (Thompson *et al*. 2004) (Fig. 1-2). Although not standardised with regard to size, the term was used to describe plastic debris that was not readily visible to the naked eye. Although still under debate, microplastic size classes were defined by Frias and Nash (2019) as "*Microplastics are any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 μm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water"*. The mass of microplastics in the environment was estimated as 400 times greater than that of macroplastics in a study of the Belgian continental shelf (Van Cauwenberghe *et al*. 2013). Subsequently, numerous reports have documented the prevalence of microplastics and their distribution in various environments. Microplastics ubiquity has been reported on in a large number of studies for example: in marine environments (Pagter *et al*. 2020b; Martin *et al*. 2017), freshwater systems (Akdogan *et al*. 2023; Murphy *et al*. 2022; Mani and Burkhardt-Holm, 2020) atmospherically (Kyriakoudes and Turner, 2023; Dris *et al*. 2016), and in terrestrial soils (Tian *et al*. 2022). Microplastics have been documented in various species (Deoniziak *et al*. 2022; Joyce *et al*. 2022; Nelms *et al.* 2019) in mountain ranges (Allen *et al*. 2019) and in regions and species of the deep sea (Courtenen-Jones *et al*. 2020; Courtene-Jones *et al*. 2019).

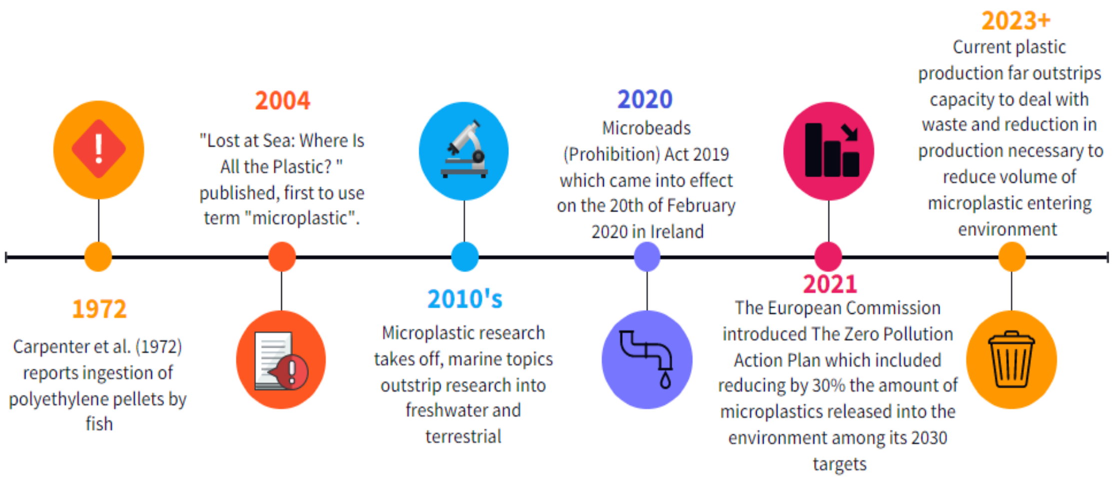


Figure 1-2: A timeline of microplastics research, legislation and current environment status.

Microplastics found in the environment have one of two classifications; primary or secondary. Microplastics can be further characterised by their physical appearance and come in a variety of forms. The shape of microplastics can sometimes give an important indication of its origin or source. Where pellets are prevalent the area receives inputs from industrial processes, fibres are indicative of residual waters from items of clothing while fragments and other types generally come from the breakdown of larger plastic items which can occur on beaches or the sea's surface (Ugwu *et al*. 2021). The size range of plastics observed in the environment compared to living organisms is shown below (Fig. 1-3).

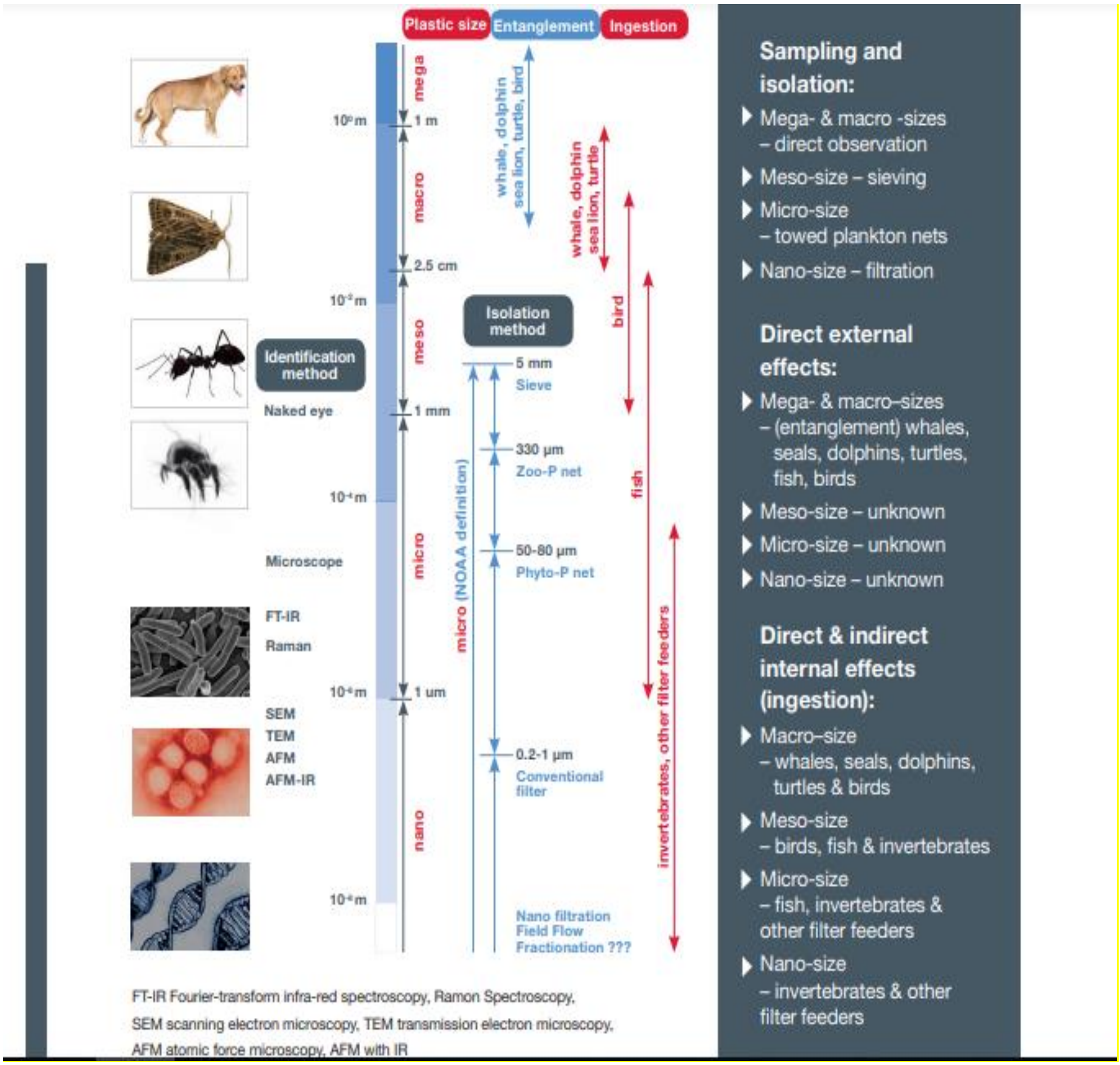


Figure 1-3: Size range of plastic objects observed in the marine environment and some comparisons with living material and overview of associated sampling methods and effects on biota (GESAMP, 2015).

1.1.3 Primary Microplastics

Primary Microplastics are described as pieces of plastic that are microscopic in size and have been manufactured specifically to be of these size ranges (typically less than 5mm) (Cole *et al*. 2011). GESAMP described microplastics based on their origin with preproduction pellets, microbeads, micro-sized powder and drug delivery all classed as primary microplastics (GESAMP, 2015). Microplastics that have been produced intentionally (primary microplastics) include; virgin pellets or preproduction pellets, microbeads that are present in cosmetic products, abrasives used in air/water-blast cleaning and powders that may be used as inks, injection molding or medicine (Rio Mendoza and Balcer, 2018). Some examples of microplastics present in brands of facial cleansers are displayed below (Fig. 1-4, Tanaka and Takada, 2016)

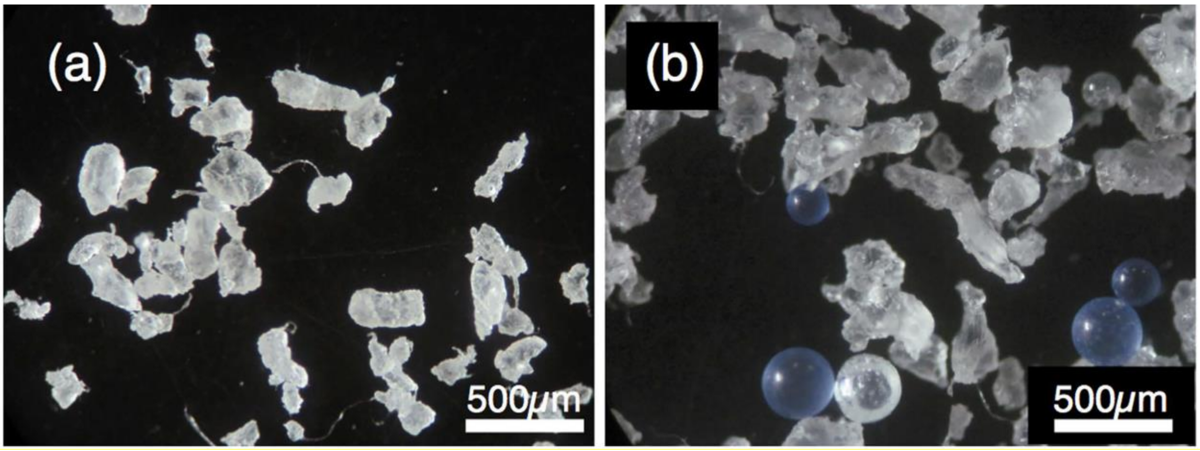


Figure 1-4: Examples of primary microplastics (polyethylene) in facial cleansers (a) transparent, irregular shapes (b) transparent and blue microspheres and transparent irregular shapes. (Tanaka and Tanada, 2016).

1.1.4 Secondary Microplastics

Secondary microplastics are tiny plastic particles that originate from the degradation and fragmentation of larger plastic litter in the environment (both at sea and on land) which can occur through various methods such as Ultraviolet (UV) radiation or mechanical abrasion (Rochman *et al*. 2013; Ryan *et al*. 2009; Thompson *et al*. 2004; Andrady, 2003). Secondary microplastics that end up entering the marine environment are more likely to stem from the degradation of macroplastics on land (beaches, etc.) than in the water body themselves where exposure to both UV radiation and mechanical erosion is minimal (Gregory and Andrady, 2003). In addition to UV radiation from the sun, plastic debris on beaches experience relatively high degradation rates due to abrasion action of waves and damage from rocks, stones and sand as well as high oxygen availability which all encourage fast rates of plastic degradation (Andrady, 2011; Barnes *et al*. 2009). Andrady (2015) underlined four key processes that help to degrade or fragment plastic material in the environment; solar UV-induced photodegradation reactions, thermal reactions including thermo-oxidation, hydrolysis of the plastic polymer and microbial degradation. Of these four processes only the first is particularly effective at breaking down plastic particles and is limited to plastics floating on the ocean's surface and on beaches (Cooper and Corcoran, 2010). At higher ambient temperatures there is a marked increase in degradation rates for plastics as their activation energies for oxidation degradation are quite low (Tocháček and Vrátníčková, 2014; Hamid and Pritchard, 1991). Plastics lying on beaches therefore undergo increased rates of photooxidation compared to those floating on the surface of water bodies due to the fact they are not surrounded by the cooler water temperatures. (Andrady, 2015). Factors that can degrade plastics in marine environments are displayed below. (Table 1-1).

Table 1-2: Comparison of the availability of weathering agents in the different zones within the marine environment (Andrady, 2015).

Weathering agent	Land	Beach	Surface water	Deep water or sediment
Sunlight	Yes	Yes	Yes	No
Sample temperature	High	High	Moderate	Low
Oxygen levels	High	High	High/moderate	Low
Fouling (screens solar radiation)	No	No	Yes	Yes

Floating plastics in aquatic environments can also undergo biofouling by algae and invertebrate species initially (Fazey and Ryan, 2016) (Fig. 1-5). This covering of the surface area can further reduce the rate of degradation by UV radiation of the plastic. If heavy fouling occurs the density of the plastic may become greater than the surrounding seawater which results in sinking, subsequent grazing may occur on the fouling species residing on the plastic causing it to move back up in the water column as its density decreases. This slow cyclic 'maritime life-cycle' of floating plastic debris was confirmed by Stevens *et al*. (1996) and Stevens (1992). Biofouling of microplastics can result in increased density which can be problematic when performing density separations for microplastic extraction from environmental matrices. However, observations made by Amaral-Zettler *et al*. (2021) on density changes due to biofouling on polyethylene postulated that sinking due to microbial biofilms alone may be more prevalent in slow moving freshwaters than in marine environments as oscillations used to mimic movement at sea dislodged loosely attached clumps of cells and the polymer became buoyant again in laboratory experiments.

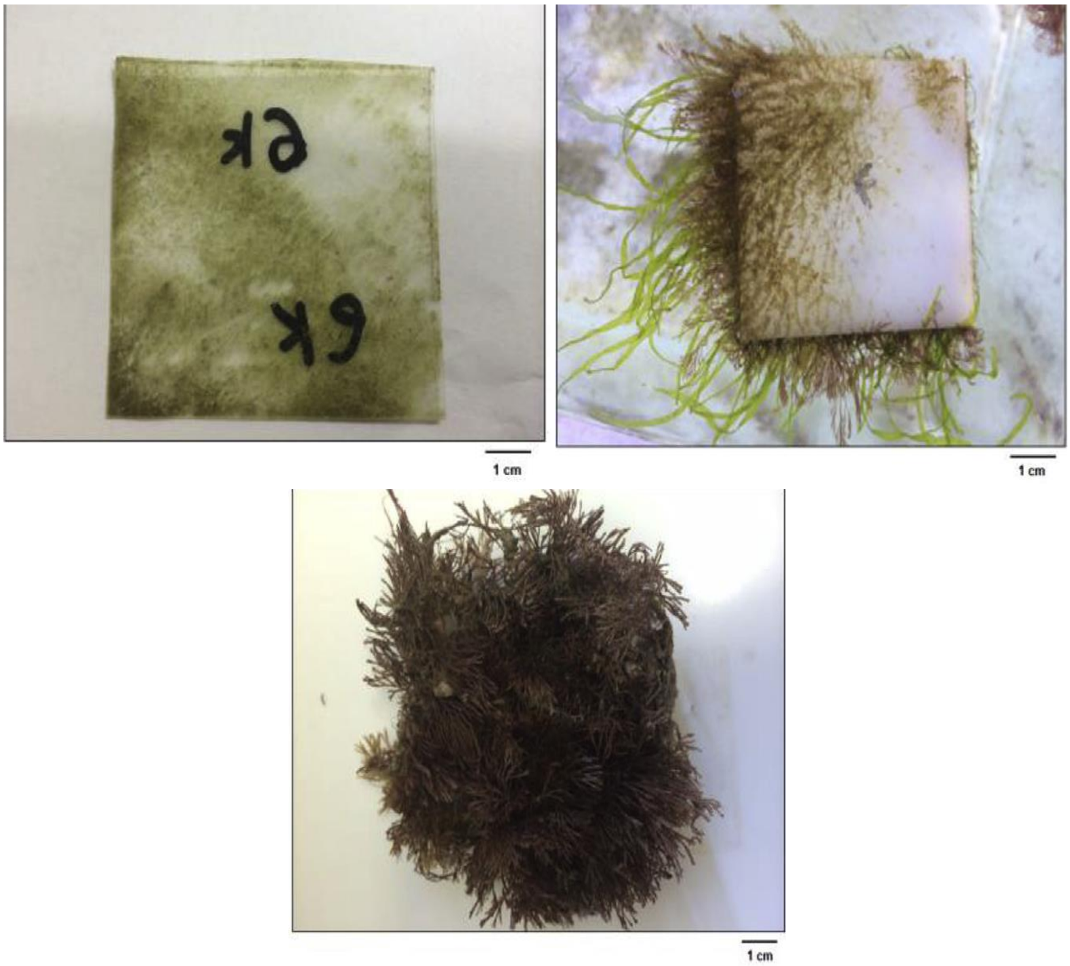


Figure 1-5: Fouling of Polyethylene after 2 weeks, 4 weeks and 12 weeks respectively in False Bay, South Africa. (Fazey and Ryan, 2016).

Degradation rates of plastics is a variable that changes greatly between beaches, surface waters of both saltwater and freshwater bodies and benthic regions in marine environments however innate properties of specific plastics will also contribute to rates of degradation. For example, the presence of UV stabilisers in processed plastics help to extend their longevity in the environment and reduce breakdown rates (Andrady, 2011). Due to the ongoing weathering (albeit slow in some cases) of plastics in the marine environment there exists a huge variety in size, shape, colour and polymer type among secondary microplastics because they can originate from the breakdown of any large plastic item (Setälä *et al*. 2018).

1.2 Interactions of Microplastics in the environment; freshwater and transport pathways

1.2.1 Rivers as transporters of plastic litter

To date, the majority of papers written on the subject of microplastics are primarily focused on marine ecosystems. A literature review conducted by Cera *et al*. (2020) found that from 2012 to 2020 2864 papers were published on microplastics and marine ecosystems while just 158 were based on freshwater ecosystems. Prior to this less than 4% of studies related to microplastics were reportedly associated with freshwaters (Lambert and Wagner, 2018). Rivers act as important transporters of plastic waste from the terrestrial environment to the marine one with the quantities transported expected to increase in the future (Jambeck *et al*. 2015). It is estimated that 1.15 to 2.41 million tonnes of plastic enter the ocean via river transport every year and that 122 polluting rivers contributed more than 90% of the total plastic inputs (Lebreton *et al*. 2017). In some instances, macroplastics and microplastics can become trapped in riverine or lacustrine environments and thus can affect these ecosystems (Ghinassi *et al*. 2023; Hengstmann *et al*. 2021). Freshwater bodies can act as sources, transporters (rivers), and sinks (isolated lakes, sediment of rivers) of microplastics and therefore high variabilities in microplastics abundances can be expected (Klein *et al*. 2018). It is estimated that only 2% of primary microplastics in the ocean were the result of marine anthropogenic activities with the other 98% the result of land-based activities (Boucher and Friot, 2017). There exists a huge range of microplastic concentrations reported in freshwater environments with a review of 183 studies by Lu *et al*. (2021) noting that microplastic concentrations span eight orders of magnitude in freshwater (1.2×10^{-3} to 5.42×10^{5} particles/m^3) and six orders of magnitude in sediments (8.1×10^{-1} to 9.5×10^{5} particles/kg).

Furthermore, around 80% of marine microplastics enter the ocean through riverine transport (Mani *et al*. 2015). Primary microplastic emissions from mainland China alone were estimated at 737.29 Gg in 2015 with one sixth of this entering the aquatic environment (Wang *et al*. 2019). Modelling work on European rivers found that the majority of microplastics exported by rivers to seas are from tyre and road wear particles (42%) and fibres (polyester etc.) (29%) which are shed from items of clothing during washing (Siegfried *et al*. 2017). For example, the mass release of tyre and road wear particles was estimated for the Seine watershed at 1.8kg inhabitant^{-1} yr^{-1} (Unice *et al*. 2019). Another source of microplastics to freshwater environments include storm water run-off which can dump microplastics from land-based activities such as agriculture into freshwater systems which is known to be a large contributor of microplastics to waterways. It is therefore unsurprising that urban development close to or on freshwater rivers leads to a high abundance of microplastics in these rivers and their sediment (Shruti *et al*. 2019; Peng *et al*. 2018). Notably, higher microplastic abundances were detected in freshwater downstream of cities with high populations in The Laurentian Great Lakes of the United States (Eriksen *et al*. 2013).

1.2.2 Wastewater Treatment Plants: sources and sinks of microplastics

While there are many potential sources of terrestrial microplastics entering freshwater bodies one particularly well-studied source are wastewater treatment plants (WWTPs) (e.g., Ridall *et al*. 2023; Montecinos *et al*. 2022; Becucci *et al*. 2021). Kay *et al*. (2018) studied microplastic levels upstream and downstream of six WWTPs and found elevated levels of microplastics downstream which was attributed to the discharge of treated sewage into the rivers, the study also reported variations in the microplastic levels which seemed to correlate to the population equivalents served. Though WWTPs can remove the majority of microplastics from influent water prior to effluent release with removal efficiencies up to 95% in some instances (Talvitie *et al*. 2017*;* Talvitie 2015) and tertiary removal reported as removing an overall 87.3% to above 99.9% of microplastics (Tang and Hadibarata, 2021), many millions of microplastics are capable of still escaping from WWTPs and entering freshwater or marine bodies (Sun *et al*. 2019). Field observations of WWTPs showed that river discharge was an important medium for microplastics releases from the terrestrial environment to the ocean (Schmidt *et al*. 2017). Furthermore, there is an apparent difference in the shapes of microplastics that are captured by WWTPs. Ben-David *et al*. (2021) noted that microplastic removal from raw wastewater can be as high as 97%, however, fibres were less likely to be captured during treatment accounting for only 74% of total microplastics in raw wastewater but accounting for 91% of microplastics in treated effluent.

Sludge generated from WWTPs is applied to agricultural land in many countries and therefore can enable the release of microplastics originally trapped by the WWTP processes to be released into the environment and is a significant source of microplastics post-treatment (Mahon *et al*. 2017).

1.2.3 Microplastics and precipitation

Precipitation has been associated with microplastic pollution in freshwater bodies in a number of studies. Microplastics were documented in rainfall over the Rocky Mountains indicating rainfall was a source of microplastic pollution of freshwater bodies (Wetherbee *et al*. 2019). Concentrations of microplastics in freshwater bodies have been reported to increase due to precipitation (Hitchcock, 2020; Xia *et al*. 2020; Wong *et al*. 2020; Schmidt *et al*. 2018), however, negative relationships between the wet season and microplastic abundances possibly due to dilution effects have also been recorded (de Carvalho *et al*. 2021; Barrows *et al*. 2018). Additionally, atmospheric transportation and deposition of microplastics has been studied as a contributor of microplastic abundances in freshwater bodies (Zhang *et al*. 2020).

1.2.4 Freshwater sediment and microplastics

Freshwater sediment can serve as both a sink and source of microplastics in the natural environment. In dry seasons due to low flow rates, higher microplastic numbers may be present in sediment rather than in surface waters as they settle (Mbedzi *et al*. 2020; Eo *et al*. 2019). Conversely, in wet seasons lower concentrations of microplastics in river sediments have been noted following flood events that may wash and resuspend microplastics present in the sediment (Liu *et al*. 2019; Hurley *et al*. 2018). The relationship between surface water and sediment microplastic abundance is an unclear one (Talbot and Chang, 2022). Microplastics in sediment may remain there for longer periods of time and represent long-term concentrations (Ding *et al*. 2019; Rochman, 2018). Liu F. *et al*. (2019) found no relationship between microplastics from sediment samples and land use for stormwater retention ponds whereas significant relationships were identified for land use and microplastic concentrations in water samples (Liu S. *et al*. 2019).

1.2.5 Population pressures and relationship to microplastics in freshwater bodies

Population pressures also play an unclear role in microplastic abundances in freshwater sediment. Microplastic abundances in river sediments in densely populated areas of Shanghai exhibited levels higher than those from sparsely populated areas (Peng *et al*. 2018). A similar trend was observed in the sediment in rivers of the Tibet Plateau (Jiang *et al*. 2019). Conversely, two Irish locations in upland areas with low population densities had higher microplastic abundances in sediment than three other locations with greater population pressures (Murphy *et al*. 2022). It has been pointed out that there is no simple relationship between either population density or WWTP proximity (Tibbetts *et al*. 2018). Additionally, current studies on microplastic pollution of freshwater sediment are concentrated in densely populated areas and those that are generally economically developed which may be leading to a bias in assessing microplastic pollution and that remote locations may be just as polluted but are currently understudied (Yin and Zhao, 2023).

1.3 Interactions of Microplastics in the Marine Environment

Microplastics whose density is greater than that of seawater may sink down into sediments and accumulate there whereas those with low densities float on the surface (Alfaro-Núñez *et al*. 2021; Frias *et al*. 2020). However, those less dense will eventually sink as a result of biofilm formation (Lobelle and Cunliffe, 2011), expelled as faecal pellets (Cole *et al*. 2013) or through the process of flocculation (Michels *et al*. 2018) meaning that marine sediment is considered the ultimate sink of many pollutants (Woodall *et al*. 2014). Furthermore, it is suggested that polymer density alone is not the most significant control on microplastic particle fate within aquatic environments (Razeghi *et al*. 2021). Although microplastics have been recorded in many marine environments, they are particularly problematic in coastal locations due to the proximity of potential sources from the terrestrial environment and also tidal processes that can encourage their deposition and accumulation (Gray *et al*. 2018; Weinstein *et al*. 2016; Ryan *et al*. 2009). Microplastic abundances in marine environments display high variability which besides the differences in environments can stem from differences in sampling methods, separation techniques and in reporting units (Lindeque *et al*. 2020; Shim *et al*. 2018). Marine sediment is classed as a major sink for microplastics with a conservative estimate of 14 million tonnes thought to reside on the ocean floor (Barrett *et al*. 2020). Several studies have documented the presence of microplastics in deep-sea sediment (Barret *et al*. 2020; Cunningham *et al*. 2020; Van Cauwenberghe *et al*. 2013b). Deep-sea sediment samples of the Indian Ocean, Atlantic Ocean and Mediterranean Sea was shown to have contamination levels up to four orders of magnitudes greater than in sea surface water samples (Woodall *et al*. 2014) which highlighted this environ as the long-term ultimate sink for microplastic debris.

1.3.1 Coastal Environs

Near-shore or intertidal habitats are known as sites of potentially high microplastics contamination given their proximity to terrestrial environments where as much as 80% of marine litter originates (European Environment Agency, 2023). Additionally, recent modelling work indicates that approximately 77% of positively buoyant marine plastic litter stemming from land-based sources spend 5 years beached or floating in coastal water (Onink *et al*. 2021). Furthermore, degradation and fragmentation of plastic into microplastic form is expected to be greatest in surface water and on beaches where the rate of solar UV-induced photodegradation is greatest (Cooper and Corcoran, 2010) and beaches are the most likely source of secondary microplastics in marine environments (Kataoka and Hinata, 2015; Kataoka *et al*. 2013; Andrady, 2011). Indeed, microplastic pollution has been noted in a large number of marine intertidal locations (Perfetti-Bolaño *et al*. 2022; Mendes *et al*. 2021; Bucol *et al*. 2020). Microplastics have been documented in coastal and marine environments in numerous recent studies and are found in varying concentrations worldwide (Fig. 1-6, Nunes *et al*. 2023). Intertidal locations in Ireland displayed a range of 0 to 553 particles per kilogram (Mendes *et al*. 2021). Across Europe microplastic contamination has been found in various concentrations in recent studies. Concentrations of microplastics of 53 ± 7.6 items per kilogram have been reported from the Black Sea which are similar to those recovered from the South-East of Spain, 64.06 ± 8.95 particles per kilogram (Terzi *et al.* 2022; Bayo *et al*. 2019).

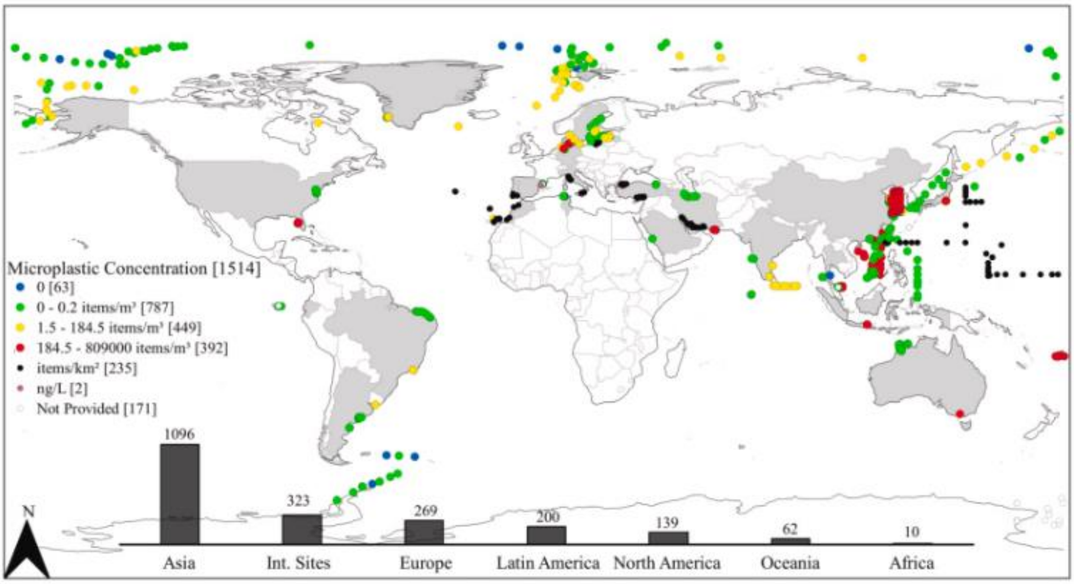


Figure 1-6: Presence of microplastics reported in coastal and marine waters worldwide (Nunes *et al*. 2023).

1.4 Microplastics and their interactions with biota

Two hundred and twenty species are noted to ingest microplastics in the natural environment (Lusher *et al*. 2017). There are numerous problems faced by biota ingesting microplastics. Following ingestion, microplastics can accumulate in an organism's organs, cause mechanical obstruction preventing feeding and can illicit effects such as false satiation effecting the energy levels in individual animals (Anbumani and Kakkar, 2018; Jovanović, 2017). Furthermore, microplastics are not homogenous particles, each differs in chemical composition both in terms polymer type and additives present, for example, phthalate can be up to 50% of the weight of PVC (Rochman, 2015). Due to their hydrophobic nature microplastics may absorb and accumulate contaminants from the natural environment (e.g., PBDEs (Polybrominated Diphenyl Ethers), EDCs (Endocrine Disrupting Chemicals), and PPCPs (Pharmaceutical and Personal Care Products), along with other Persistent Organic Pollutant (POPs) in aqueous media which may then desorb when conditions become favourable such as being ingested by an organism (Martín *et al*. 2022; Joo *et al*. 2021). Indeed, up to 81 different chemical compounds have been found in microplastics in the Canary Islands (Camacho *et al*. 2019). Furthermore, greater ingestion of microplastics in seabirds has been linked to chemical aromatic signal from dimethyl sulfide release in microplastics (Savoca *et al*. 2016) while an increase in epithelial cysts was noted in plastic-feeding birds (Roman *et al*. 2019).

1.4.1 Bioturbation and microplastics

Bioturbation or the processes by which animals alter their habitats by influencing the sediment structure of soft bottoms of the sea floor include actions such as burrowing, ingestion, defecation and ventilation which transport particles in the sediment matrix (Kristensen *et al*. 2012). Several microcosm experiments have demonstrated the vertical transportation of microplastics into deeper sediment layers by various species (e.g. the wedge clam *Donax trunculus*, the Baltic clam *Macoma balthica*, the lugworm *Arenicola marina* and the brittlestar, *Amphiura filiformis*) (Ben-Haddad *et al*. 2022; Coppock *et al*. 2021; Gebhardt and Forster, 2018; Näkki *et al*. 2017). Benthic species can therefore lead to the increased burial and sedimentation of microplastics in the marine environment (Coppock *et al*. 2021). The cumulative effect of macrofauna communities is the net burial of microplastics (Coppock *et al*. 2021).

1.4.2 Microplastics and their entry to food webs

Zooplankton play a crucial role in the food chain as a major link between primary producers and higher trophic levels (Ikeda, 1985). Zooplankton are a wide-ranging group of primary and secondary consumers that includes both vertebrates and invertebrates such as microscopic copepods, fish larvae and jellyfish which consume free-floating algae and other zooplankton. The role that zooplankton play in marine food webs cannot be overlooked as they form a bridge for energy transfer between primary producers (phytoplankton) and larger predators which are themselves then preyed upon by predators further up the food chain. For zooplankton microplastics represents a nutritionless by-product that is ingested alongside other food sources leading to a reduction in primary producer consumption and their associated nutrients by zooplankton (Kvale *et al*. 2021). Because of their key role in the uptake of energy into food webs it is important to understand the possible impacts and implications that the presence of microplastics in aquatic environments can have on their health. Zooplankton, in addition to energy transfer within food webs, are also key to the biological carbon pumping that drives photosynthetically fixed organic carbon from the surface to the intermediate and deeper oceans and water bodies (Turner, 2015; Steinberg and Landry, 2017; Turner, 2002; Longhurst and Glen Harrison, 1989). Feeding in surface waters, zooplankton produce faecal pellets which sink and may be sequestered or remineralised by other zooplankton and through vertical migration can return to waters at the surface (Turner, 2015). Coastal waters are highly productive and biodiversity-rich ecosystems which are home to diverse species of zooplankton (Anandavelu *et al*. 2020), however, the likelihood of exposure to microplastics for organisms in these environments is enhanced (Cózar *et al*. 2014). Microplastic abundance is higher here due to the proximity of coastal waters to the terrestrial environment and where fishing pressure is expected to be greatest both of which are sources of microplastic pollution to coastal waters (Clark *et al*. 2016). Due to the fact that zooplankton are consumed by higher organisms, they are potentially a starting point for microplastics entering the diets of a variety of species including humans (Fig. 1-7). Furthermore, modelling work has shown that zooplankton grazing on microplastics could decrease water column oxygen inventory by as much as 10% in the North Pacific exacerbating the consequences of climate warming (Kvale *et al*. 2021).

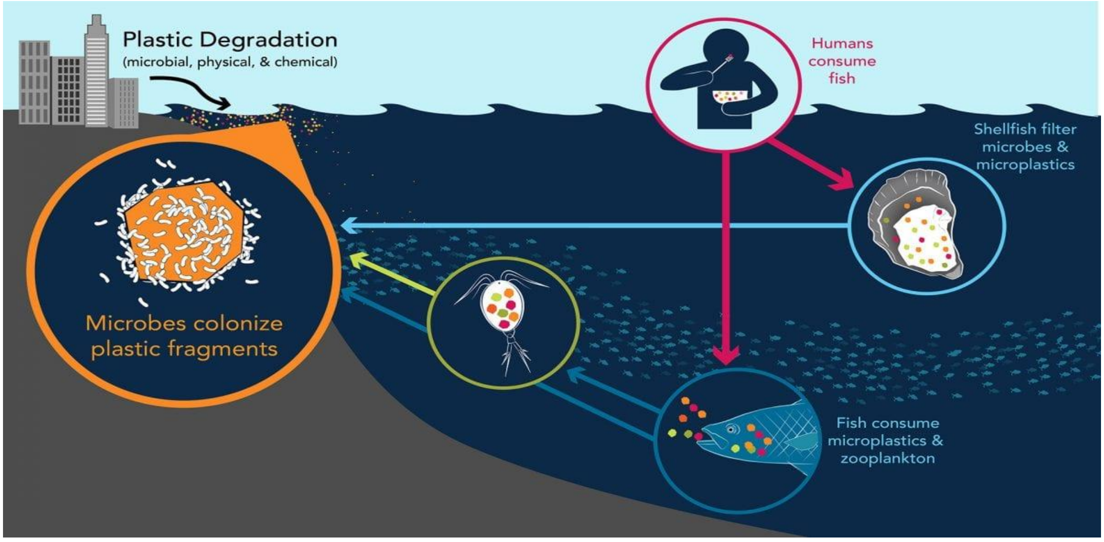


Figure 1-7: The pathway that zooplankton may introduce microplastics to the diets of a wide range of species on higher trophic levels including humans (Lubofsky, 2018).

1.5 Microplastics: more than plastics, associated effects

Aside from the physical issues that can arise following ingestion of microplastics such as blocking of the gastrointestinal tract and false satiation, other issues can arise from the leaching of chemicals present in the microplastics themselves. Plasticisers (additives) are chemicals added to plastics during production to give them desirable properties for the role they will have in industry as well as helping to extend their lifespans. Some effects plasticisers can have include; thermal resistance (e.g. PBDEs), increased flexibility and durability, buoyancy aids and microbial resistance (e.g. Triclosan) (Thompson *et al*. 2009; Browne *et al*. 2007). Unfortunately, these properties also increase the effect that plastics can have on the environment as degradation times are increased and the leaching of these additives can occur, some of which can have undesirable effects on biota. Many additives to plastics are classed as EDCs, two examples of which are Bisphenol-A (BPA) and Phthalates. Human exposure to endocrine disrupting chemicals can result in; birth defects, neurodevelopment conditions, reproductive health impacts, obesity and metabolic diseases (OECD, 2023). In animals the effects can be much more wide ranging, some responses to EDCs in animals include but are not limited to; imposex (masculinisation) of female sea snails, egg-shell thinning in sea birds, effects on the immune system and reproductive organs, as well distorting of sex organs and functions among alligators (Vos *et al*. 2000).

1.6 Viruses and microplastics

Biological substances can potentially proliferate on the surface of microplastics or indeed after being ingested or inhaled by organisms together with microplastics (Guo *et al*. 2020; Lobelle and Cunliffe, 2011) and therefore the potential of biological impacts on organisms from microplastics may be of greater concern than chemical concerns associated with microplastics. In humans however, this is a new research pathway of microplastic study. A variety of diseases capable of infecting humans have been shown to survive days and even weeks on plastic surfaces (Moresco *et al*. 2021; Rzezutka and Cook, 2004). In laboratory studies aged microplastics showed better protection of the virus, *Escherichia coli* bacteriophage T4 in comparison to pristine microplastics which may be due to increased surface roughness enabling increased survival of the virus (Lu *et al*. 2022). WWTPs may have potential as breeding grounds for microplastic coated in viruses that can be discharged into downstream aquatic environments given how they discharge both a large amount of microplastics (Sun *et al*. 2019) and viruses (Corpuz *et al*. 2020). Notably, viruses such as rotavirus, hepatitis A and norovirus are shed in high concentrations in the faeces of infected individuals and are commonly detected in raw sewage, treated effluents, sludge or the surface waters of receiving water bodies (Farkas *et al*. 2018*;* Iaconelli *et al*. 2017; Prado *et al*. 2019; Schlindwein *et al*. 2010).

1.7 Microplastics entering the human diet

Dietary exposure of microplastics to humans is currently an area of prominent research as the health effects of these pollutants on humans are unknown. However, commonly contained additives can have detrimental effects and some polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) are carcinogenic in nature. Commonly-consumed food products such as table salt, sugar, honey, beer, water, edible fruits and vegetables have been reported to contain microplastics (Oliveri Conti *et al*. 2020; Renzi and Blašković, 2018; Liebezeit and Liebezeit, 2014,2013) and drinking water has been suggested as the main source of microplastics to human diets (Senathirajah *et al*. 2021). Another well-studied entry route of microplastics to human diets is the consumption of seafood, in particular, bivalves. Bivalves are important filter-feeding organisms in many marine environments in addition to forming part of the diet of many invertebrate and vertebrate species at different stages of their life cycle. Bivalves also provide an important protein source for many people. Bivalves (primarily clams, mussels and oysters) accounted for 16 million tonnes of coastal and marine animal aquaculture in 2015) (FAO, 2016). Bivalves are a group of animals particularly at risk to the effects of microplastic exposure due to the fact they feed through the process of filtration and prey size similarity (Germanov *et al*. 2018; Wright *et al*. 2013). The presence of microplastics in surrounding water filtered by bivalves may result in their ingestion and illicit negative responses in the individual. The ingestion of microplastics by bivalves is worthy of study as in general the entire bivalve is consumed and thus raises concern for human health implications of microplastics, furthermore bivalves are consumed by many different marine species which can exacerbate their proliferation in marine and freshwater food webs (Fig. 1-8). Beyond bivalves, microplastics have been documented in organisms at several trophic levels and some examples are displayed below (Table 1-3).

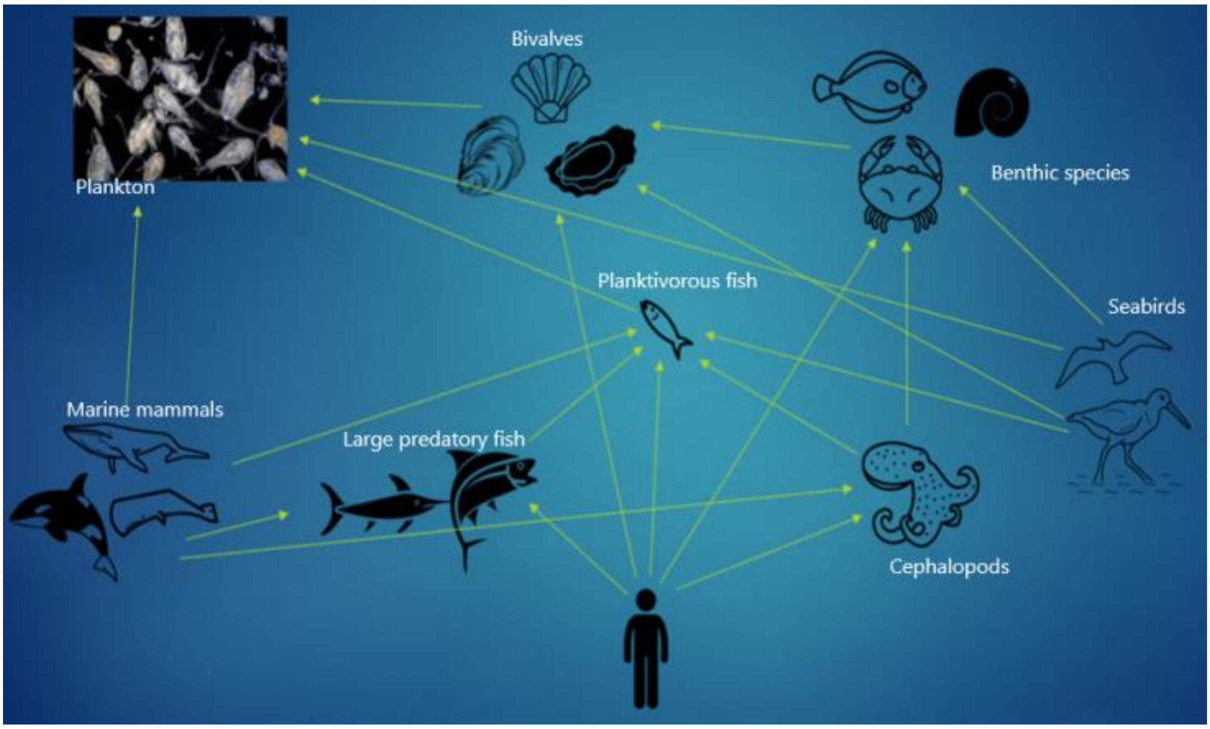


Figure 1-8: Diagram displaying the potential predator-prey transfer network of microplastics in marine food webs and the many routes that humans may be exposed.

Table 1-3: Microplastics documented in organisms at different trophic levels.

Organism	**Reference**
Zooplankton	Botterell *et al*. 2022; Goswani *et al*. 2023; Cole *et al*. 2013.
Seabird (*Hydrobates pelagicus melitensis*)	De Pascalis *et al*. 2022
Seabirds (14 species)	Navarro *et al*. 2023

Bivalvia: *Meretrix meretrix*[1], *Tegillarca granosa*[2], *Perna viridis*[2], *Chlamys farreri*[3], *Mytilus galloprovincialis*[3], *Crassostrea gigas*[3], *Ruditapes philippinarum*[3]	Wu *et al*. 2022[1]; Ta *et al*. 2022[2]; Ding *et al*. 2021[3]
European pilchard (sardine) (*Sardina pilchardus*), Gilt-head bream (*Sparus aurata*), Striped red mullet (*Mullus surmuletus*), Common sole (*Solea solea*)	Ferrante *et al*. 2022
Swordfish (*Xiphias gladius*), Bluefin tuna (*Thunnus thynnus*)	Di Giacinto *et al*. 2023
Human Placenta	Zhu *et al*. 2023
Human Blood	Leslie *et al*. 2022
Human Lungs	Jenner *et al*. 2022
Human testis and semen	Zhao *et al*. 2023

1.8 Widely reported microplastic presence in bivalves

In recent decades much like other species of marine life there has been a reduction in some population sizes of bivalves (FAO, 2016) with species of freshwater bivalves among the most threatened with extinction groups in the world with 40% of the species being near threatened, threatened or extinct (Lopes-Lima *et al*. 2018). Microplastics in marine and freshwater environments present a relatively new threat to these species. A growing body of evidence indicates the ubiquity of microplastic contamination of bivalves (e.g., Aung *et al*. 2022; Baechler *et al*. 2020; Hermabessiere *et al*. 2019).

Bivalves filter water for nutrients at different rates and exist in a variety of sizes (both species dependent). Bivalves, in particular species of mussels have been commonly used as sentinel organisms to monitor any anthropogenic pollution in marine coastal environments in which they commonly inhabit (Li *et al*. 2016; Goldberg *et al*. 1978) and so the examination of bivalves for microplastics may give an indication into levels of microplastics in aquatic ecosystems. Oysters, in addition to mussels are deemed ideal test organisms for the monitoring of environmental pollutants due to their high accumulation of a wide range of these pollutants coupled with their sessile lifestyle (Xie *et al*. 2016). Although capable for use as biomonitoring tools for microplastic pollution on a regional basis the lack of harmonisation in studies hinders the effectiveness of comparing data on a global scale (Ding *et al*. 2022) in addition the huge number of variables must be considered when comparing data sets on bivalve microplastic pollution. The physiology of bivalves can be affected by the ingestion of microplastics; oysters (*Grassostrea gigas)* displayed changes in their feeding capacity and reproductive output when exposed to polystyrene microspheres (Sussarellu *et al*. 2016).

Differences between numbers of microplastics reported between studies is not unexpected. Variation in microplastics concentrations between species of bivalves can depend on several environmental factors including but not limited to; location in the water column of the studied species, waste management of the nearby terrestrial area and proximity of studied bivalves to sources of microplastic pollution. However, several other factors can also lead to variation between results such as; the chosen digestion method of bivalve soft tissue, filtration mesh size chosen post-digestion, whether bivalves are allowed to filter clean water before examination and also in reported units used (items/gram or items/individual).

1.8.1 Associated effects of microplastics to bivalves

Body condition indices have generally been the target of microplastic ecotoxicity studies on bivalves in the past, however there has been no noted impairment of these indices in several species including; *Scrobicularia plana* (Ribeiro *et al*. 2017), *Ennacula tenuis* and *Abra nitida* (Bour *et al*. 2018), *Mytilus edulis* (von Moos *et al*. 2012), *Crassostrea gigas* (Sussarellu *et al*. 2016), *Cerastoderma glaucum* or *Limecola balthica* (Urban-Malinga *et al*. 2021). Because of the little or no effects on body condition noted in ecotoxicology studies, bivalve body condition has been generalised as an insensitive marker of microplastic ecotoxicity (Bour *et al*. 2018). Microplastics however can impact the behaviour of bivalves living in sediment which in turn can lead to knock-on effects for benthic environments. The cockle, *Cerastoderma glaucum* which is a near-surface dwelling species emerged less often and in lower numbers from sediment spiked with microplastics while the Baltic clam, *Limecola balthica* buried deeper in similarly treated sediment than in controls with no microplastic presence (Urban-Malinga *et al*. 2021). The majority of microplastic contamination studies into both ‘wild’ and ‘farmed’ bivalves have been carried out in Asia and Europe (Ding *et al*. 2022), Asia being the largest producer of marine bivalves by far accounting for 85% of the worldwide market (Wijsman *et al*. 2019).

Global aquaculture production stood at 110.2 million tonnes in 2016 with 17.1 million tonnes coming from molluscs (FAO, 2018) and between 2009-2014 marine bivalves accounted for about 14% of the global marine production. In contrast to other fish types bivalves are generally consumed whole meaning that any microplastics ingested and present in the gastrointestinal tract (GIT) will be consumed by humans. As mentioned previously, bivalves are particularly susceptible to ingestion of microplastics due to their extensive filter feeding, exposing them not only to potential microplastics in zooplankton but also to any microplastics in the water column and sediment itself.

1.9 Taking Action: UN Sustainable Development Goals

The current trends of pushing the nine safe planetary boundaries for existence on Earth are unsustainable. Novel entities of which plastics are described as a particular subset of high concern of chemicals are being produced and emitted at increasing rates and volumes into the environment. This production rate is currently outstripping any efforts at safety assessment and monitoring and are transgressing the planetary boundary with immediate action needed to return us to a safe operating space (Persson *et al*. 2022). Trajectories show that global plastic use is projected to triple between 2019 and 2060 from 430 Mt to 1,312 Mt (OECD, 2022). Furthermore, the chemicals associated with plastics are also largely unregulated with health consequences known for only some of them and over 5,000 academic papers have been published which describe plastic-related harms to human health focused on phthalates, flame retardants and bisphenols (Merki and Charles, 2022). Two recent reviews of industrial, scientific and regulatory data carried out to assess the number of chemicals used in plastics (Aurisano *et al*. 2021; Wiesinger *et al*. 2021) identified 13,000 chemicals potentially used in plastics. Of these 13,000 chemicals, 3,200 were identified as chemicals of potential concern based on existing hazard types (UNEP, 2022) and problematically 6,000 chemicals had no hazard data available thus illuminating the issues associated with exponential plastic production and inferior disposal and management methods. The reviews also found that just 128 chemicals of concern are regulated under multilateral environment agreements (Fig. 1-9).

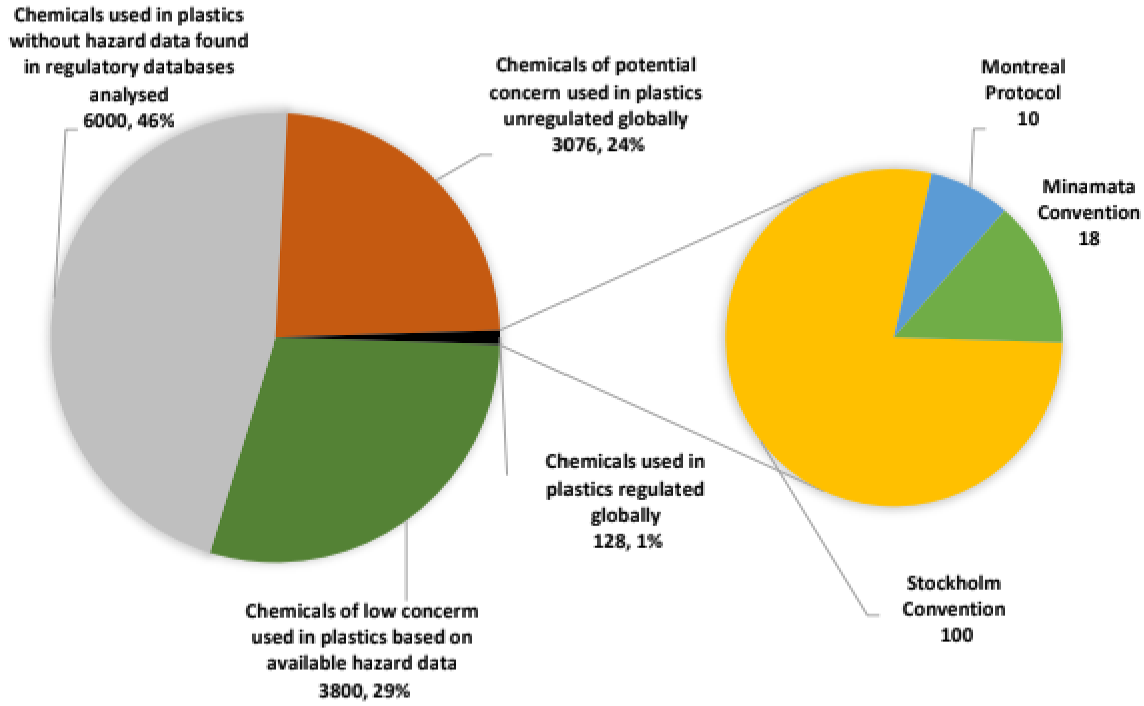


Figure 1-9: Number of chemicals of concern found in plastics as per reviews of Wiesinger *et al*. (2021) and Aurisano *et al*. (2021). Produced by UNEP, 2023.

In 2015, the United Nations adopted the Sustainable Development Goals (SDGs) as a universal call to action to end poverty, protect the planet and ensure that by 2030 all people enjoy peace and prosperity. There are 17 SDGs that are integrated together and actions on one SDG will impact the others. While there has been no explicit consideration of microplastics in any of the 17 UN SDGs, microplastics pollution directly or indirectly impacts at least 12 UN SDGs and it has become more needed than ever to evaluate the human and ecological health impacts of these pollutants as well the threats to environmental, social and economic sustainability (Walker, 2021). A growing body of evidence shows that microplastics accumulate in organs and tissues in aquatic organisms causing impaired development, oxidative stress, inflammation, neurotoxicity and intestinal injuries (Iheanacho *et al*. 2023) and affects their behaviour when present in their environment (Urban-Malinga *et al*. 2021). Additionally, and perhaps most troubling of all is that microplastics as outlined earlier have recently been detected in human faeces (Schwabl *et al*. 2019), lung tissue (Jenner *et al*. 2022), semen and testis (Zhao *et al*. 2023), placentas (Ragusa *et al*. 2021) and breast milk (Ragusa *et al*. 2022) while the health effects of microplastics on humans are currently unknown.

While not explicitly stated, two SDGs are particularly and implicitly associated with the microplastics issue; 12 and 14. SDG 12 deals with responsible consumption and production while SDG 14 is life below water. Key goals of SDG 12 that can lead to reduced microplastic losses and entry into the environment via human behaviour include; that 1. by 2030 waste generation will be substantially reduced through prevention, reduction, recycling and reuse. 2. by 2020, achieve the environmentally sound management of chemicals and wastes throughout their life cycle and 3. by 2030, ensure that people in all countries have relevant information and awareness for sustainable development and lifestyles that are in harmony with nature. From SDG 14, key points relating to addressing the microplastics issue are; by 2025, that marine pollution of all kinds is prevented and significantly reduced, especially from land-based activities, including marine debris and nutrient pollution. By 2020, sustainably manage and protect marine and coastal ecosystems to avoid significant adverse impacts, via strengthening their resilience and take action towards their restoration in order to achieve healthy and productive oceans, and to increase scientific knowledge, develop research capacity and transfer marine technology in order to improve ocean health and to enhance the contribution of marine biodiversity to the development of developing countries, in particular small island developing states and least developed countries. The role of managing plastic production, waste and pollution in attaining the aforementioned SDGs cannot be understated. It was estimated that in 2016 between 19-23 Mt of plastic waste generated reached aquatic ecosystems but this is predicted to reach 53 Mt by 2030 (Borelle *et al*. 2020). It has been demonstrated that the plastic life cycle contributes to climate change and biodiversity loss and is outside the safe operating space of the planetary boundaries (Persson *et al*. 2022), additionally, plastic waste and pollution costs up to US$2.5 trillion per year based on reduced ecosystem services (Beaumont *et al*. 2019).

As seen from the above SDG 12 is intrinsically linked to the outcomes of SDG 14. For example, from SDG 12, a reduction of plastic use would lead to a reduction of plastic waste, which would reduce the amount reaching landfills or the natural environment such as marine systems directly, potentially impacting biota and environs found within, both in macrolitter or microliter form. Therefore, in order to successfully achieve the goals of SDG 14 current human behaviour, attitudes and awareness must be assessed in relation to SDG 12 as these will give an understanding of shifts needed by society and the modified behaviours which can protect freshwater and marine environments. Currently downstream strategies exist that mitigate plastic pollution reaching the environment but these are viewed as ineffective in the face of current plastic production and pollution (Walker and Fequet, 2023). Waste generation outpaces existing regulations and removal methods and this is particularly problematic in developing countries (Ferronato and Torretta, 2019). The majority of consumer plastics are designed for single-use applications with limited recyclability thus leading to huge production and increasing consumption trends and therefore waste generation (Borrelle *et al*. 2020; Lau *et al*. 2020). Given this "throwaway culture" which has been so prevalent since the mass production of plastics began. It is important to examine the psychological aspects and behaviour of consumers which currently exist such as their motivation for using plastics, how they dispose of them, how they view them and what they know about the harmful aspects of plastics after they are disposed of. In order to deal with mismanaged plastic waste, the waste hierarchy can be followed and comprises five steps: prevention, reuse, recycling, energy recovery and disposal (Diggle and Walker, 2020). Following this, a reduction in plastic use would supersede efforts to reuse or recycle currently outstripped by plastic production and consumption rates. The transition from unsustainable plastic production and consumption rates to renewable products will require substantial changes in behaviour for industry, government and consumers over the coming years (Walker and Fequet, 2023). This is a pertinent issue in the Republic of Ireland currently. The Minister for the Environment, Climate and Communications of Ireland, Eamon Ryan, speaking on the Sustainable Development Goals at the United Nations in New York noted that while Ireland had achieved 80% of its associated targets it was "*disappointing to see that we are missing targets related to what should be quite basic issues in a developed society, like municipal waste and consumption*" and acknowledged the need for greater renewable energy rollout (Department of the Environment, Climate and Communications, 2023).

1.10 Legislation and policy on plastics and microplastics

In order to limit the introduction of waste material into the marine environment from terrestrial sources the implementation of measures through combining existing and new legislation as identified under waste strategies and marine litter action plans is the focus of descriptor 10 of the Marine Strategy Framework Directive (2008/56/EC). Through the increase of reuse and sustainability incentives (from the EC (Waste Directive) regulations and Waste Management Act Directive 2008/98/EC) which include treating waste as a resource, the amount of litter that is generated on land will be reduced. This in turn will limit the amount of waste that may enter the marine environment or end up on coastlines around Ireland through river transport or blowing from coastal landfills. The reduction in litter generation, will ultimately reduce the introduction of secondary microplastics into the marine environment, which is accelerated on coastlines and beaches (Andrady, 2011; Barnes *et al*. 2009). In broader terms the European Green Deal is designed to make the European Union climate neutral by 2050 which includes decoupling resource use from economic growth which in turn will lead to a reduction in plastic use, tackling plastic packaging use and its replacement with more sustainable alternatives (European Commission, 2021).

Human behaviour has been identified as a major factor that is fundamentally linked to awareness, perception, attitude, level of concern about marine litter, in addition to motivations to engage in solutions to address this issue (Hartley *et al*. 2015; Rees and Pond, 1995). On a larger scale, different factors such as policies and legislations can influence behaviour which can benefit or degrade the environment (Beeharry *et al*. 2017). While the majority of society will in general dispose of litter in a responsible way this can depend on the environment they are in and the behaviour of others. Various studies have shown that people are more likely to litter in an already littered environment compared to a pristine one and are also less likely to litter having witnessed someone else picking up litter (Bator *et al*. 2011, Keizer *et al*. 2008*;* Cialdini, 2003,1990). It has been noted that improved anti-littering behaviour at a personal level could positively impact the behaviour of the larger social system (Beeharry *et al*. 2017). Currently, measures are being taken to reduce microplastic pollution with national governments banning products such as microbeads and single-use products. At an international level the United Nations has made commitments to reduce plastic entering the environment through addressing single-use plastic products pollution, the UN Environment Assembly Resolution Marine Litter and Microplastics and the UN Sustainable Development Goals (SDGs) (Walker, 2021). It is increasingly obvious that system change is required rather than decisions by individuals in order to stem the amount of plastic waste being discharged into the environment as production currently outstrips any recycling / reusing / refilling infrastructure available.

In 2018, the governments of the European Union, Canada, Germany, Italy, the United Kingdom and France signed the 'Ocean Plastic Charter' during the 45th summit of the G7 aiming to prevent plastic waste and unnecessary use of single-use plastics while simultaneously promoting recycling and research into plastic alternatives (Canada; Environment and Climate Change, 2018). By working with industries and adopting specific policies they aimed to reach: i) 100% recyclable or recoverable plastics by 2030; ii) by 2030 at least 50% of recycled content in plastic items; iii) at least 55% of recycled packaging by 2030 and 100% of all plastic recovery by 2040 and iv) a reduction in the use of plastic microbeads.

Microbeads from personal care products and more recently microfibers from clothing are a main focus of microplastic research as levels of concern are beginning to increase amongst the general public over these microplastics. This in turn has led to increased legislation and policies targeting this particular group of pollutants with microbeads in particular the target of new legislation.

Microbeads were banned in Ireland under the Microbeads (Prohibition) Act 2019, which came into effect on the 20th of February 2020. This legislation prohibits production of personal care products, cosmetics and cleaning products that contain microbeads in addition to banning the import or export of said products while making it an offence to dispose of products containing microbeads into drains or aquatic environments (EPA, 2019). This legislation follows a similar banning in the United Kingdom of microbeads in 2018 (The Environmental Protection (Microbeads) (England) Regulations 2017 No. 1312). This ban does not include products that protect from UV light such as sunscreen which are still allowed to contain microbeads. This may be an oversight as products that contain microbeads such as cosmetics that end up washed down drains in homes at least have the potential of capture in WWTPs while microbead-containing sunblocks may be washed from the body while in the marine environment directly entering the ecosystem of coastal regions. Other countries that have introduced bans on microbeads include; the US, Canada, New Zealand, France, India, Sweden and Taiwan (Fig. 1-10). Microbeads have been banned in personal care products such as gels, toothpastes and scrubs since 2015 in the Netherlands (Fela, 2014) and since 2018 in the U.S (H.R.1321 - Microbead-Free Waters Act of 2015). Bans of this nature are direct measures to stop the production of microplastics used in certain products reducing the amount of primary microplastics that can impact on the environment and are examples of cutting plastic production which are measures that have been called for by many (Bergmann *et al*. 2022). Although the ban on microbeads is a positive step it is a very small one relative to the amount of microplastics that enter waterbodies from other sources.

The tackling of the microplastics issue is not only an issue that should be dealt with by the Irish government, it is also one that must be tackled under EU law. Descriptor 10: Marine Litter of the European Union's Marine Strategy Framework Directive (2008/56/EC) states that: properties and quantities of marine litter do not cause harm to the coastal and marine environment and furthermore that the amount of litter and its degradation products are reducing overtime along coastlines and the marine environment. This descriptor must be met by every member state with marine waters (including Ireland) in order to achieve “good environmental status” (GES) in their marine waters by 2020 (European Commission, 2008). The European Commission introduced The Zero Pollution Action Plan published in May 2021 which includes reducing by 30% the amount of microplastics released into the environment among its 2030 targets (European Commission, 2021). Following the publication of the ‘Annex XV dossier’ by the European Chemicals Agency which estimated that currently, more than 42,000 tonnes of intentionally-present microplastics are released into the environment each year, the European Commission adopted the microplastics ban on September 25th 2023 to restrict intentionally added microplastics to products (ECHA, 2023). The restriction would comprise synthetic polymer microparticles below 5 mm and fiber-like particles below 15 mm that are used in products on intention and may result in environmental release. This proposal however fails to tackle the problem of secondary microplastics and instead focuses on primary microplastics.

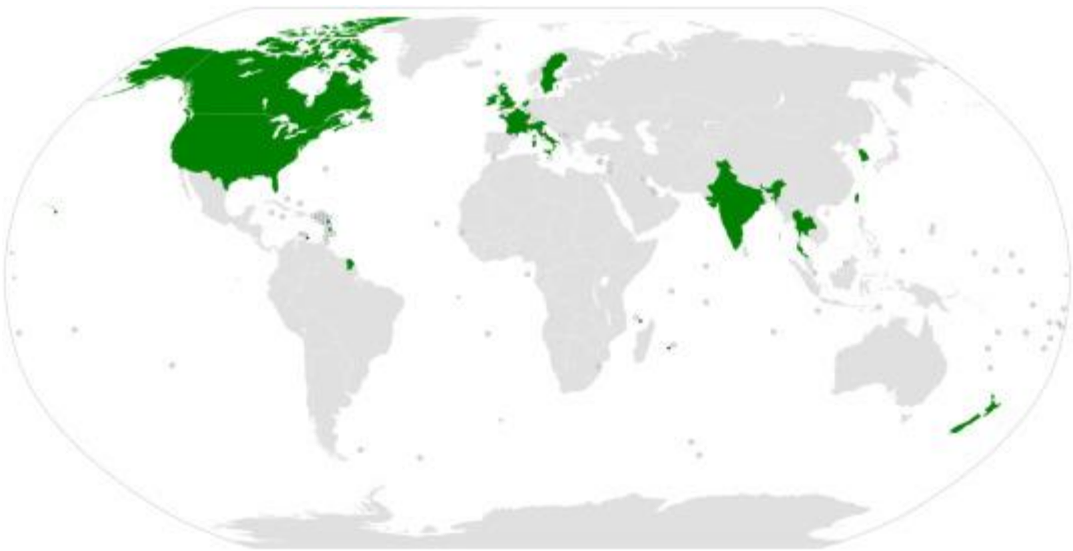


Figure 1-10: Current global microbead policy interventions showing national bans (solid green) (https://commons.wikimedia.org/w/index.php?curid=65416735).

Currently, only 9% of plastics are recycled and 79% are buried or released into the environment (Garcia and Robertson, 2017). Removal of microplastics from the environment is very difficult and on a large-scale is not feasible, therefore prevention of their release into the environment is paramount. To effectively counter microplastics entry to the environment a shift from synthetic products is needed. While this is impractical for products where plastic is the most effective material (medical use, packaging etc.) a change should be promoted to inhibit fast-fashion production and encourage natural fibre clothing procurement which would limit microplastic entry into the environment.

1.11 Thesis aims and objectives

The overall aim of this study was to investigate the abundance and impact of microplastic contamination on the freshwater and marine environments, communities and economies of Dundalk Bay and Catchment.

Specific research objectives were as follows:

1. Quantify and characterise microplastic contamination in surface waters and macroinvertebrates of freshwater river systems flowing into Dundalk Bay over a 2-year period (Chapter 3).

2. Assess the microplastic contamination in coastal sediments and in the commercially and ecologically important marine bivalve, *Cerastoderma edule* (common cockle) and note seasonal variations (Chapter 4).

3. Employ an habitat-level approach to assess microplastic pollution level within the lower levels of the marine web of Dundalk Bay. Microplastic concentrations between species will be assessed for intertidal and subtidal species that represented different feeding mechanisms in order to ascertain which are most at risk of microplastic pollution in the Bay and their potential serviceability as a biomonitor for microplastics (Chapter 5).

4. Gain insights into the relationship of community stakeholders, Irish fishers with plastic use, and microplastic contamination (Chapter 6).

1.12 Thesis outline

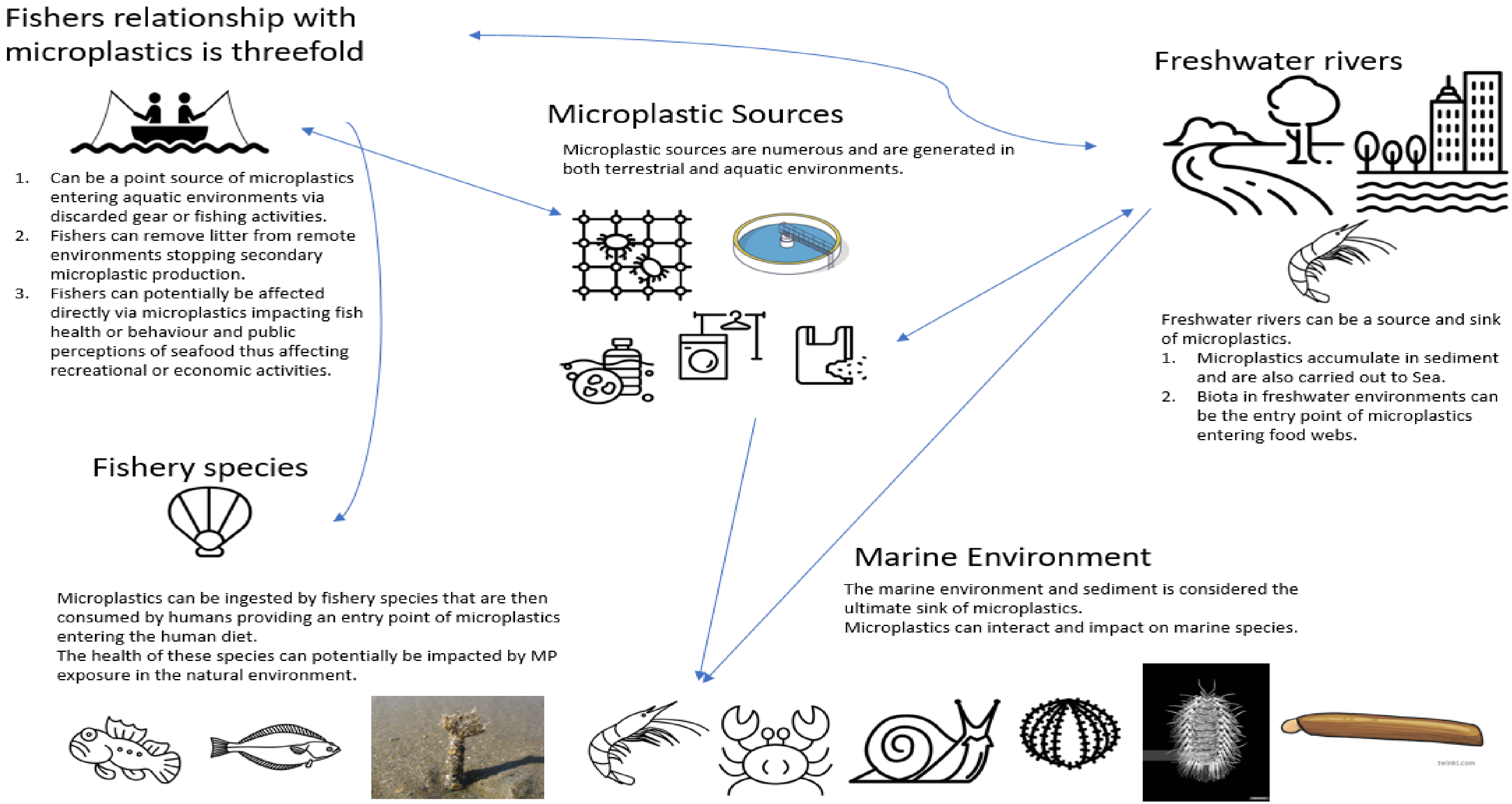


Figure 1-11: Overview of thesis structure and connections between chapters

Chapter 2: Review of microplastics isolation and identification procedures

2.1 Diversity of methods used in microplastics research

Given the increase in the last decade of microplastics research there has been a simultaneous increase not just in the methods used to isolate microplastics from environmental matrices but also in the reporting units which makes comparing studies difficult if not impossible. At least eight different units are used when describing the numbers of microplastics found in water and sediment (Lu *et al*. 2021) highlighting the lack of standardisation within the research field, while several reviews have also highlighted this variation (Lusher *et al*. 2020; Underwood *et al*. 2017; Wesch *et al*. 2016). It has been noted that the rush to generate new and more detailed data and establish baselines for microplastics contamination has not occurred in synchronisation with the development of comparable methods and there has been an exponential increase in new methods used from 2011 in particular (Rist *et al*. 2021) (Fig. 2-1). Furthermore, control methods must be implemented to minimise any microplastic contamination of samples and blanks must be carried out to quantify contamination that may have been introduced due to laboratory procedures (Hermsen *et al*. 2018). Many steps may be taken to identify microplastics from environmental samples and a general stepwise procedure is displayed below (Fig. 2-2).

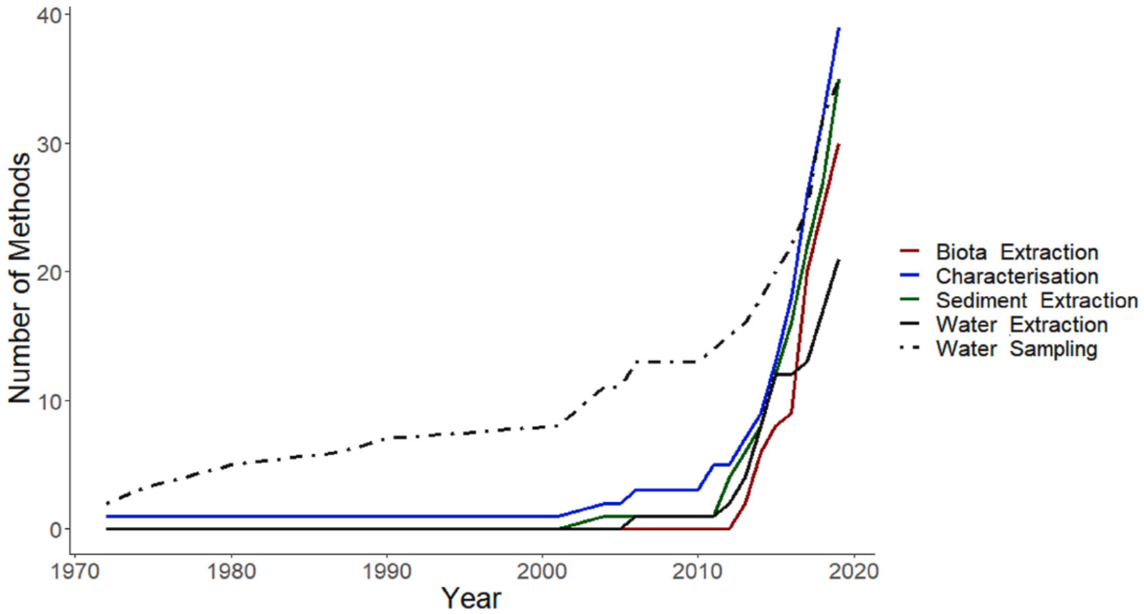


Figure 2-1: Annual increase of entirely, partially or somewhat novel methods in extracting microplastics from Rist *et al*. (2021).

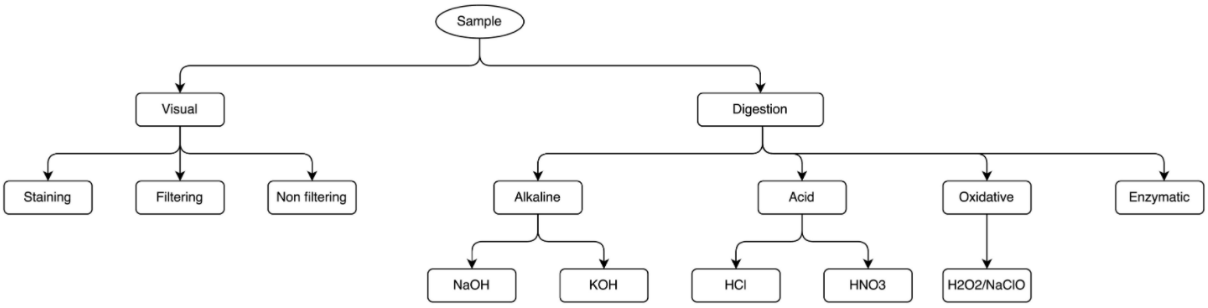


Figure 2-2: Diagram showing commonly used approaches for examination and extraction of microplastics from environmental samples from Budimir *et al*. (2018).

2.2 Water sampling for microplastics

Across freshwater sampling campaigns for microplastics there is a significant lack of uniformity. Differences exist in; sampling methodology, reporting units, digestion methods and a great deal of heterogeneity is often quoted as a difficulty in comparing results (e.g., Elert *et al*. 2017; Hidalgo-Ruz *et al*. 2012; Prata *et al*. 2019; Mai *et al*. 2018). Differences in units used for reporting of microplastics varies between studies and results are affected by the different protocols used, for example, micro particles per litre and particles per km^2 are incomparable units used for surface water microplastics content.

Furthermore, there is a wide range of microplastics reported in freshwater bodies ranging from almost none to several million cubic pieces per cubic metre and these differences can result from factors such as sampling locations, human activities, natural conditions and sampling approaches (Eerkes-Medrano *et al*. 2015). Water samples for both fresh and saltwater environments are collected similarly using three main groups including; trawls, pump samplers and bulk samples, each with their own pros and cons, however, differences in water densities may lead to distinct differences in microplastic distributions in each environment (Prata *et al*. 2019). Additionally, among water types studied reported microplastic concentrations differ widely and the fact that studies can specifically target different size classes contributes to this variability (Koelmans *et al*. 2019). Trawls and nets are generally deployed from boats in the middle of a water body and towed behind the vessel, additionally they can be hung from bridges that cross rivers and are two commonly used volume-reduced sampling methods. The area sampled is calculated by multiplying the towing distance by the trawl width. A review of sampling methods by Rist *et al*. (2021) found that net towing was the dominant method used for water sampling used in 63% of studies, however, variation exists among this method with 16 net types noted. Towing of neuston and manta nets (333µm) can sample near-surface and surface water respectively. Plankton nets (100µm) are generally towed at a lower speed as they can clog quicker. Mesh size of the selected nets plays an important factor both in composition and concentrations of microplastics recovered from water samples. Vermaire *et al*. (2017) revealed concentrations almost a hundred times higher when using a nylon net over a manta net when sampling water from the Ottawa River. Water samples taken in the Danube River using a 500µm mesh led to much lower microplastic concentrations than other studies using mesh seizes of approximately in 330µm mesh at other locations (Lechner *et al*. 2014; Su *et al*. 2016; Anderson *et al*. 2017). Additionally, when an 80µm mesh was used 100,000 times the amount of marine litter was reported than when a 450µm mesh was used (Lozano and Mouat, 2009). Furthermore, an 80µm mesh has been shown to filter 250 times more fibres than a net with a 330µm net (Dris *et al*. 2018a). Plankton nets can recover concentrations of microplastics 30 times higher than manta nets (Dris *et al*. 2015). While an increased number of microplastics will be recovered using a smaller meshed nets they can only be deployed for a small amount of time due to their clogging from suspended organic and inorganic material present in the water (Prata *et al*. 2019) and therefore can only filter a smaller volume of water than nets with larger mesh sizes. The review of microplastic sampling methods by Prata *et al*. (2019) further documents the advantages and disadvantages between methods currently in use to sample microplastics form sediment and water (Table 2-1).

Pumps can also be used to retrieve water samples either on-shore or on-board a research vessel, however, issues can arise with regards to the volume sampled and also require a power source (Tamminga *et al*. 2019; Dris *et al*. 2018b; Desforges *et al*. 2014). Alternatively, water sampling can be done via bulk sampling using bottles, containers or buckets and one quarter of water sampling studies reviewed by Rist *et al*. (2021) used this methodology. Filtration is an extra step that is necessary to carry out following bulk water collection, however, it allows the retention of microplastics smaller than the standard aperture of the mesh used in net tows or sieve stacks used in pump sampling (Enders *et al*. 2015; Law and Thompson, 2014; Mai *et al*. 2018).

Sieve stacks used with bulk water sampling on-site in order to obtain a greater volume than collecting samples to return to the lab. While this method can be labour intensive and suitable for small-medium water volumes the use of sequential sieves can aid in sample refinement and is useful for locations where sampling using trawling is impossible (Prata *et al*. 2019). For example, the majority of rivers sampled as part of achieving research objective 1 (chapter 3) for this thesis were too small for a boat to access meaning equipment was carried to sites. Using sieve cascades of different mesh sizes for filtering water samples allows for size separation and quantification of different size classes of microplastics (Löder and Gerdts, 2015).

Table 2-1: Methods to sample microplastics in water and sediment from Prata *et al*. (2019) review.

Sample	Type	Advantages	Disadvantage
Water	Neuston and Manta nets	Easy to use; Sample large volumes of water; Largely used (good to compare between locations); Produces large numbers of microplastics for further testing.	Expensive equipment; Requires boat; Time-consuming; Potential contamination by vessel and tow ropes; Lower limit of detection is 333 µm.
	Plankton net	Easy to use; Lowest limit of detection 100 µm; Quick to use; Samples medium volumes of water.	Expensive equipment; Requires boat; Static sampling requires water flow; May become clogged or break; Sampling of lower volumes of water than Manta trawl.
	Sieving	Does not require specialized equipment nor boat; Easy to collect samples.	Laborious and time consuming; Samples medium volumes; Manual transfer of water with buckets
	Pumps	Samples large volumes of water; Effortless; Allows choice of mesh size.	Requires equipment; Requires energy to work; Potential contamination by the apparatus; May be difficult to carry between sampling locations.
	Filtration or Sieving *ex situ*	Easy to collect samples; Known volume of water; Allows choice of mesh size.	Sampling of low volumes; Transportation of water samples to the lab; Potential contamination by the apparatus; Time consuming depending on mesh size.
Sediment	Beach sediment collection	Easy to implement; Rapid sampling; Allows collection of large volumes of sample or replicates.	Variation with sampled area and depth.
	Seabed collection (Grab sampler, box corer, gravity core)	Easy to use; May allow replicates.	Expensive equipment; Requires boat; Variation with sampled area and depth; Sampling may disturb sediment surface.

2.3 Sediment sample collection

Sediments are considered long-term sinks of microplastics (Rochman, 2018; Li *et al*. 2022). Plastics greater in density than the surrounding water body will sink while those lighter shall float on the water's surface or in the water column (Andrady, 2011). However, the density of floating plastics can increase due to processes such as; biofilm formation and the adsorption and accumulation of pollutants can result in microplastic presence in sediment (Van Cauwenberghe *et al*. 2015a; Xu *et al*. 2020; Lobelle and Cunliffe, 2011). Just as difficulties exist in comparing water microplastic abundances due to sampling methodologies, reporting units and sample sizes so too are similar differences restricting comparisons of microplastic abundances in sediment (Ivleva *et al*. 2017) and the need for standardisation exists.

Freshwater and marine sediment has been collected with a wide range of sampling tools dependant on the purpose of the study. In general, three different types are used. Corers to take depth samples generally of the bottom sediment of the water body or beaches where the objective of the study is to track historic presence of microplastics (Turner *et al*. 2019; Vaughan *et al*. 2017). Grabs or grab samplers to collect surface sediment samples from the river or sea floor and will provide data on the most recent deposition of microplastics on the riverbed (Alam *et al*. 2019; He *et al*. 2020; Rodrigues *et al*. 2018). Spades, shovels, spoons, trowels, spatulas etc. (Jiang *et al*. 2019; Klein *et al*. 2015; Mani *et al*. 2019) can collect samples from the riverbank and marine beaches which is used to obtain data that can reflect long-term interfacial interaction between waters and the terrestrial environment (Yu *et al*. 2016). A review of 38 studies on freshwater sediment microplastic pollution by Yang *et al*. (2021) noted a wide variety of sampling unit used. Approximately half of the studies reviewed used the sampling tool area as the sampling unit, varying from 250 cm^2, 400 cm^2 to 930 cm^2 while other studies used volume (1 – 3.5L) or weight of sediment (0.2 -5kg) (Yang *et al*. 2021). Selecting an appropriate field sampling method will depend on the sediment matrix and the microplastic size distribution targeted (Waldschläger *et al*. 2022). Plastic tools should be avoided in particular when sampling for sediment as friction of sharp sediment grains with the sampling tool can cause abrasion and can increase in microplastics in the sediment (Adomat *et al*. 2022).

2.4 Assessing microplastics in biota

The ubiquity of microplastics in marine and freshwater bodies has led to increased concern for the ecological consequences of this new and varied pollutant on both a species and ecosystem-level. This has led to a renewed interest in biota monitoring for the accurate determination of microplastics abundances in oceans (Yusuf *et al*. 2022). Just as certain species have different sensitivities to different elements (Zawadzki *et al*. 2016) species may have preferential ingestion, retention and encountering rates with microplastics dependent on factors such as feeding mechanisms, size and their position in aquatic environments or trophic levels (Walkinshaw *et al*. 2020; Sun *et al*. 2019; Botterell *et al*. 2019). Due to these factors special attention should be given to the biota selected depending on the aims of the study to give a realistic overview of microplastic pollution in an ecosystem. Bessa *et al*. (2019) reported a set of protocols for effective selection of the appropriate species that have the potential for acting as sentinels for microplastic monitoring in the aquatic environment:

- Species that occur naturally in high abundance with a wide geographical range.

- Species that are easily sampled and controlled in laboratory settings (e.g. macroinvertebrates).
- Species already used in biomonitoring in different research relative to marine pollution.
- Species that have socio-economic and ecological relevance.
- Species that serve several functional / ecological roles, niches, feeding guilds.

The use of endangered / protected species for microplastics research is not advisable and ethical guidelines and requirements should be followed (Bessa *et al*. 2019). For analysing microplastics in larger animals it is often ideal to assess plastic ingestion without the death of the animal (e.g., making birds regurgitate their stomach contents (Provencher *et al*. 2019) or by assessing it in animals that had died through other circumstances (Nelms *et al*. 2019).

Species at lower trophic levels in general can be adequate indicator species for monitoring of pollutants due to their position of at the base of food chains (Von Moos *et al*. 2012). The monitoring of benthic communities is common practise for environmental impact assessments in marine environments and these have been used to assess the prevalence of disturbance and pollution (Bilyard, 1987), heavy metals (Kress *et al*. 2016), nutrient loading (Naser, 2010) and oil spills (Lee and Lin, 2013).

Shellfish and crustaceans that are consumed whole are particularly well-studied (Ding *et al*. 2022) due to their potential of transferring microplastics to humans. In aquatic environments, the most commonly studied organism in the literature for monitoring microplastics is the blue mussel *Mytilus edilus* (Vandermersch *et al*. 2015). Freshwater vertebrates such as fish and birds are investigated for microplastics contamination more than microorganisms or invertebrates (Cera and Scalici, 2021). Amongst freshwater invertebrates, bivalves are the main group studied in freshwater systems. For species selection, a review by Wesch *et al*. (2016) produced four important steps that in order to conduct effective microplastics monitoring of biota which are: (1) suitable indicator species selection, (2) sampling and sample processing, (3) analytical procedures and (4) preventative measures to ensure secondary contamination of the samples does not occur. While one species has generally been used to assess microplastic pollution in the literature, Pagter *et al*. (2020a) used an ecosystem-based approach in Galway Bay which can be useful for microplastic pollution in an area devoid of sufficient numbers of suitable bioindicators. By implementing this approach, the authors explored the community as a whole, providing data for short to long-term modelling, biomonitoring and decision-making for a given area (Pagter *et al*. 2020a).

Biota are investigated in a number of ways for microplastics presence. Several species have been investigated for; total body microplastic content (Hermabessiere *et al*. 2019), casing content (Ehlers *et al*. 2020) and specific organ content or tissue (Di Giacinto *et al*. 2023). For larger animals such as bigger fish specimens, birds and sea turtles, dissection and visually sorting gastro-intestinal tract is a common approach (Markic *et al*. 2020; Janardhanam *et al*. 2022; Parton *et al*. 2020). The investigation of the gastrointestinal tract of larger animals enables an insight into the potential trophic transfer of microplastics from small species into bigger ones. If the goal of the study, however, is to quantify human exposure to microplastics via consumption then it is necessary to study the parts consumed by humans, e.g., Di Giacinto *et al*. (2023) documented microplastics in edible muscle of swordfish, (*Xiphias gladius*) and bluefin tuna (*Thunnus thynnus*). For bivalves the entire soft tissue is commonly analysed for microplastics as they are consumed whole by humans (Ding *et al*. 2022). When comparing studies examining microplastics in biota, differences in pore size of the filter paper used, digestion methods and control procedures used to isolate microplastics from environmental samples can result in differences that not neccasarily linked to environmental contamination.

2.5 Isolating microplastics from non-complex matrices

Regardless of sample matrix examined, a separation procedure is required in order to effectively quantify the presence of microplastics as biological or inorganic material can mask their detection. For bulk-water samples vacuum filters with a Buchner funnel are the most common which is carried out ex-situ (Lusher *et al*. 2020). For ‘clean’ samples such as drinking water or small volumes of surface water or those containing little biological or inorganic material (Ivar Do Sul and Costa, 2014; Lusher *et al*. 2014) no pre-treatment is generally required and these can be poured directly onto filter papers to assess microplastic presence.

Sieving is common for the extraction of microplastics from sediment either wet or dry in nature and in-situ on studies focused on plastics that can be separated by eye with sieves of 1mm, 2mm or 5mm used (Karkanorachaki *et al*. 2018). The use of sieve stacks will divide the sample into easy to manipulate sub-samples with some having less biological material present resulting in less further steps to purify and isolate microplastics (Lusher *et al*. 2020). Microfibre quanitification for samples is notably difficult. Walkinshaw *et al*. (2022) noted that fibres with lengths up to 1700µm were captured on the 25µm mesh having passed through meshes with larger pore sizes previously. It was postulated that while fibres are measured by their length they are very small in diameter and may pass through larger mesh sizes when orientated correctly (Barrows *et al*. 2017; Coverton *et al*. 2019). The same was noted for fragments greater than 100µm in size. This was explained due to particles with a large axial ratio passing through sieves with coarse meshes when orientated correctly and also due to inconsistencies across the filter in terms of pore size that may be exacerbated by pressure from a vacuum pump pulling fragments through mesh pores (Walkinshaw *et al*. 2022).

As previously mentioned, a sieve cascade can be used with bulk water sampling in-situ. This method alone is not viable where the purpose of the study is to analyse microplastics <1mm (Lusher *et al*. 2020), it may have applications for citizen science projects however. For example, dry sieving was used to quantify microplastics in marine sediments from the Irish continental shelf using interlocked sieves of 250µm, 400µm and 500µm and found to be superior than density separation using sodium polytungstate (SPT), however 250µm was the lower limit investigated (Martin *et al*. 2017).

2.6 Density separation techniques

Whilst sieving can remove larger organics it will not separate microplastics from material of a similar size range (Nabi *et al*. 2022). In samples that contain biological material or large amounts of inorganics further sample clarification is needed to quantify microplastics effectively. Density separation procedures are routinely used in separate microplastics from sediment, digested biota and material trapped via water sampling, however the choice of salt solution can vary (Fok *et al*. 2020; Möller *et al*. 2020). Super-dense solutions are made by dissolving salts into water in which plastics that are less dense than the solution will float in. The density range of polymers can range; 0.85 g cm^{-1} for PP to 1.37 g cm^{-1} for PET encompassing LDPE (0.91 g cm^{-1}), HDPE (0.94 g cm^{-1}) and PS (1.05 g cm^{-1}) (Omexus). Salts commonly used to make brine solutions are sodium chloride (NaCl) (e.g., Di and Wang, 2018; Frias *et al*. 2016), sodium iodide (NaI) (e.g., Claessens *et al*. 2013; Di and Wang, 2018; Ling *et al*. 2017) and zinc chloride ($ZnCl_2$) (e.g., Liebezeit and Dubaish, 2012), Rodrigues *et al*. 2018; Horton *et al*. 2017) while other salts are used to a lesser extent (e.g., calcium chloride, zinc bromide) (Cutroneo *et al*. 2021). Additionally, sodium tungstate dehydrate has also been used to effectively separate microplastics from marine sediments and is safer to use than other salts (e.g. $ZnCl_2$, NaI) (Pagter *et al*. 2018). There are positives and negatives associated with each salt to be considered prior to their selection for research work. Associated negatives can be the environmental impacts, toxic nature, cost, reduced recovery etc. (Mani *et al*. 2019; Nabi *et al*. 2022). Salts which have the highest recovery of plastics are also the most expensive (e.g., $ZnCl_2$ and NaI) which can prove unfeasible for projects with smaller budgets (Rodrigues *et al*. 2020). For example, the high cost of NaI limits its use for significant volumes of sediment (Claessens *et al*. 2013). To reduce associated costs, the reuse of these solutions on subsequent samples is suggested (Rodrigues *et al*. 2020). $ZnCl_2$ and NaI can be made up to form saturated solution with a density of 1.8 g cm^{-1} (Coppock *et al*. 2017; Willis *et al*. 2017), whereas NaCl is used to make up a solution of 1.2g cm^{-1}) (e.g., Bayo *et al*. 2019). Further issues arise with the use of $ZnCl_2$, while it is effective in recovering high-density plastics (e.g., PVC, PET) it is harmful and corrosive and incredibly toxic to aquatic life meaning that solutions are time-consuming to handle, cannot be used for citizen science projects and is expensive to dispose of (Nabi *et al*. 2022). $ZnCl_2$ and NaI can be 4 – 10 times more expensive than NaCl solutions (European Chemicals Agency (ECHA) zinc chloride, 2020; ECHA sodium iodide, 2020). On the other hand, commonly used deionized water and saturated NaCl solutions are effective at separating low-density polymers such as polyethylene, polypropylene and polystyrene from sediments and are cheap, readily available and comparatively environmentally friendly (Masura *et al*. 2015; Zhang *et al*. 2018). Density separation using NaCl is recommended by the MSFD technical subgroup (2013) and the NOAA (Prata *et al*. 2019). More recently, Gohla *et al*. (2021) used potassium carbonate (K_2CO_3) with a density of 1.54 g/cm^3 as a novel floating solution for microplastic isolation from beach sediments, praising its high recovery rate, non-toxic nature and cheap cost which makes it particularly promising for long term and large-scale monitoring studies. Mean recovery rates without repeated extractions were over 90% for PVC, PET, PP and HDPE, furthermore, K_2CO_3 was particularly lauded for its hazard-free workflow and trouble-free disposal which also lends to citizen science engagements, additionally, it is recyclable and can be filtered following use for future work (Gohla *et al*. 2021)

The selection of salt solution for density separation is dependent on a plethora of conditions and requirements. Additionally, the sediment type under investigation can influence the selection. For example, due to the presence of soil organic matter (SOM) the separation of microplastics from soil environments such as agricultural fields is expected to be more difficult than separating microplastics from sandy beaches as SOM can bind soil particles together and impacting the efficiency of microplastic extraction (Bläsing and Amelung, 2018; He *et al*. 2018).

When using bulk water or sediment sampling in can be useful to remove the water fraction. In order to expedite gravity settling and separation, centrifugation has been used previously. Centrifugation (3500g x 5 minutes) was used to isolate microplastics from marine sediment in Belgium (Van Cauwenberghe *et al*. 2013) and animal faeces along French–Belgian–Dutch coastlines (Van Cauwenberghe *et al*. 2015b). A 93% recovery rate was reported for centrifugation (2000g x 10 minutes) of marine sediments with density separation from Eastern Asia and South Africa (Matsuguma *et al*. 2017). Phoung *et al*. (2018) used centrifugation for the extraction of microplastics from sediment in from the French Atlantic coast. More recently, testing of various centrifugation speeds and times on different freshwater samples revealed 3500g for 5 minutes to be efficient to settle the mineral and organic material, while preserving the polymers and showing high microplastic recovering rates (93 ± 6%) (Monteiro *et al*. 2022). From terrestrial samples, Grause *et al*. (2022) achieved 94% recovery of a range of polymers from agricultural soil using centrifugation, while centrifugation was used by Wu *et al*. (2021) to isolate polypropylene particles from swine manure and achieved higher recoveries than gravity separation.

2.7 Oil separation; environmentally friendly, safe and cheap

Several novel methods have been trialled for microplastic separation from sediments. Electrostatic separation (Felsing *et al*. 2018), magnetic extraction (Grbic *et al*. 2019) and elutriation column optimisation (Claessens *et al*. 2013; Hengstmann *et al*. 2018) are all lesser used methods. Oils have seen an increase in use in recent years for microplastic extraction from sediment, soil and digested biota samples. Canola oil (Crichton *et al*. 2017; Crew *et al*. 2020), sunflower oil (Song *et al*. 2022), olive oil (Scopetani *et al*. 2020) and castor oil (Mani *et al*. 2019) have all seen use. High recovery of spiked microplastics using castor oil and the reduction of the irrelevant part of the matrices studied was noted by Mani *et al*. (2019). Following the digestion of mussel tissue using H_2O_2, Song *et al*. (2022) reported total extraction recovery rates of PP, PVC and PET ranging from 95.6 ± 5.09% - 100% using sunflower oil. A high recovery of microplastics of the polymers PS, PE, PVC, PET, PUT and PC with a range of 90 – 97% was observed from soil and compost samples when using olive oil (Scopetani *et al*. 2020). Castor oil had a mean recovery rate for a range of sizes and polymers of 99 ± 4% from marine beach sediments, agricultural soil, fluvial suspended surface solids and marine suspended surface solids (Mani *et al*, 2019).

2.8 Digestion protocols

To successfully quantify microplastics from organic-rich samples a digestion protocol is generally necessary to break down organic material. Several different methods are present in the liertature. The Food and Agriculture Organisation of the United Nations (FAO) report on microplastics in food tabulates a large amount of these various agents used for the breakdown of seafood (Gamarro and Costanzo, 2022). Digestion protocols can be tailored and selected based on the material under investigation.

2.8.1 Biological digestions

Various enzymatic digestion protocols have been employed to breakdown organic matter. Cole *et al*. (2014) utilised proteinase-K to visualise microplastics from plankton-rich seawater with a 97%< by weight digestion of material present. The use of protease is recommended as a standard method for extracting microplastics from mussel soft tissues (Catarino *et al*. 2017). Von Friesen *et al*. (2019) reported that pancreatic enzymes (PEz) in combination with (tris(hydroxymethyl)aminomethane)/Tris hydrochloride solution were effective in digesting tissue of *Serripes groenlandicus* for microplastic analysis and was superior than KOH digestion in terms of organic matter removal and time. Löder *et al*. (2017) used a mixture of enzymes (protease, cellulase, chitinase) to breakdown material recovered via surface water sampling which reduced 98.3 ± 0.1% of the sample matrix. Trypsin yielded the greatest weight reduction of *Mytilus edulis* of 3 proteolytic digestive enzymes tested with no observed impacts on microplastics (Courtene-Jones *et al*. 2016). While the treatment of biological samples with enzymes can be gentler on polymers than chemical treatments, they still have drawbacks limiting their applicability (Von Friesen *et al*. 2019). A cocktail of enzymes may be needed which can increase the cost or the digestion may take a long time (up to 15 days) (Löder *et al*. 2017). Some industrial enzymes are additionally very expensive (e.g., Proteinase-K; Cole *et al*. 2014). Furthermore, some multi-step procedures can increase the risk of sample contamination or particle loss (Lusher *et al*. 2017).

2.8.2 Oxidising agents

Hydrogen peroxide (H_2O_2) is a strong oxidising agent known to break down organic matter (Schrank *et al*. 2022). A solution of 30% H_2O_2 has been used for the digestion of filtration residues (Lin *et al*. 2018), sieved material (Zhang *et al*. 2019) and dried sediment samples (Peng *et al*. 2017). Additionally, it has been used in some studies isolating microplastics from biota (e.g., Song *et al*. 2022; Pazos *et al*. 2020; Nalbone *et al*. 2021), however a review of digestion protocols by Bai *et al*. (2022) found H_2O_2 was used the most in non-seafood studies. Combining H_2O_2 with ferric ion (Fe(II)) catalyst (Fenton reaction) can increase the efficiency of H_2O_2 digestion (Hurley *et al*. 2018; Tagg *et al*. 2017; Masura *et al*. 2015). Avio *et al*. (2015) noted that while H_2O_2 was efficacious as a digestant for the intestinal tracts of mullet (Mugil cephalus) its application led to only 70% retrieval of spiked microplastics which was linked to excessive foaming, noted in several other studies (Lusher *et al*. 2017). Furthermore, Pfeiffer and Fischer (2020) found that H_2O_2 at all tested concentrations did not yield good digestion efficiencies of the test biogenic organic material when applied at room temperature even when left for 7 days. It was noted that increasing the temperature improved digestion but an increase in temperature to 70–100°C can cause a significant loss in weight and size of PA as well as a loss of colour when applied even at room temperature (Duan *et al*. 2020; Hurley *et al*. 2018).

2.8.3 Alkaline and acidic digestion

The alkaline chemical potassium hydroxide (KOH) can be used to digest animal tissues via hydrolysis and the denaturation of proteins and has been used for digesting soft tissues of several marine biota (e.g., Pagter *et al*. 2021; Kühn *et al*. 2017; Tanaka and Takada, 2016). KOH is recommended for the digestion of biota for microplastics as it does not affect most polymer types with the exception of cellulose acetate (Dehaut *et al*. 2016; Kühn *et al*. 2017). The damage of KOH to microplastics is relatively mild when compared to treatments that use other chemicals such as sodium hydroxide (NaOH), nitric acid (HNO_3), and hydrochloric acid (HCl) (Thiele *et al*. 2019). However, KOH and NaOH are not as successful when used for digestion of sediment and water samples, as biogenic organic matter often originates from plant material (leafs, wood, algae) and contains parts of shells or carpaces (Duan *et al*. 2020). Pfeiffer and Fischer, (2020) found that acid protocols using HCl and HNO_3 were the only agents that effectively digested calcareous material in the form of shells with a reduction of 99.5 – 100% in weight of material at all concentrations and tested temperatures. However, acids are generally avoided as common plastics such as Polyamide (PA), polyester and polycarbonate (PC) have low resistance to acids, even when they are at low concentrations (Lusher *et al*. 2017). Increased temperatures and concentrations of digestion chemicals resulted in accelerated degradation of all tested polymers (Pfeiffer and Fischer, 2020). Damages associated with acid use include the fusing of PS, PP, PET, low-density polyethylene (LDPE) and high-density polyethylene (HDPE), a total loss of PA and colour changes to most polymers (Catarino *et al*. 2017; Avio *et al*. 2015; Claessens *et al*. 2013).

Thiele *et al*. (2019) recommended the use of 10% KOH at 40°C for 24 hours for the digestion of shellfish tissue based on cost, expenditure of time and potential health risk of the reagents used. Dawson *et al*. (2020) found there was no substantial changes in the chemical spectral profiles of the polymers; PE, PS, Rayon, polyethersulfone (PES)following exposure with 10% KOH at 40°C. Karami *et al*. (2017) also evaluated and recommended the use of KOH at 40°C but found that an increase to 60°C was associated with reduced recovery and surface damage of PET. Marine biota with high lipid content can prove difficult for digestion using KOH as potassium-based salts form a soft (liquid suspension) soap (Konkol and Rasmussen, 2015). Some authors have suggested that 10% KOH may be avoided for using on samples with high lipid content or fat deposits (Bessa *et al*. 2019), however, it may be necessary to study fat-rich tissues such as liver and this saponification can be overcome by the addition of ethanol (Dawson *et al*. 2020). Ethanol (EtOh) has been shown to have minimal effects on microplastics (Herrera *et al*. 2018; Courtene-Jones *et al*. 2017). Longer exposure times with KOH (4 days), have been reported to have substantial visual impacts on textile microfibres for PET, polylactic acid and modacrylic (Von Friesen *et al*. 2019) so this should be avoided when using for digestion of soft tissue of biota. Ultimately, the specific properties of samples under study should be considered and these will determine the most suitable extraction protocol of microplastics from biological tissue (Karlsson *et al*. 2017).

2.9 Microplastic identification and characterisation

It is necessary to distinguish synthetic material from organic material to confirm microplastic presence, furthermore, microplastics may be categorised by a number of different shape and colour profiles as well as by plastic type. These can help to determine the origin of the microplastics in question. In earlier studies on microplastic presence visual sorting of microplastics was the primary method used for water, sediment and biota samples and was common for larger microplastics (e.g., Shim *et al*. 2017; Lusher *et al*. 2017). However, for particles <500μm visual microscopy alone is not advised (Zhang *et al*. 2020). In order to enhance visual inspection techniques dying of microplastics can be carried out, normally using Rose Bengal or Nile Red dyes. Fluorescent staining is commonly carried out using Nile red (Erni-Cassola *et al*. 2017; Shim *et al*. 2016). While the method is fast and has a strong fluorescence signal there are drawbacks associated with its use. Notably, due to the fact the dye molecules are just physically adsorbed to the microplastic surface they can desorb easily (Lv *et al*. 2019). Furthermore, the low hydrophobicity of polymers such as PVC, PC and PET renders the dye incompatible with their staining (Erni-Cassola *et al*. 2017). Rose-Bengal stains natural and non-plastic material like cotton, which may appear similar to plastic under the microscope (Ziajahromi *et al*. 2017). Visual sorting cannot yield any chemical information of the polymers found in samples (Lavers *et al*. 2016) and the probability of misjudgements will increase when the clarity and colour of plastics have been changed during extraction or purification steps.

The most commonly used methods for polymer identification are Raman spectroscopy and Fourier-transform infrared spectroscopy (FTIR), both of which are recommended by the Marine Framework Directive Technical Subgroup on Marine Litter in European Seas (Hanke *et al*. 2013). Furthermore, it is necessary to analyse a subset of particles to determine their origin and ensure that an overestimation of microplastics is not occurring through misidentification during microscopy. In environmental samples fibres are typically the dominant shape profile of microplastics recovered and can outnumber microplastic particles of other shape profiles by a factor of 10 across habitats from freshwater to the marine (Anderson *et al*. 2017; Barrows *et al*. 2018; Horton *et al*. 2017). Additionally, microfibres can have very small diameters sometimes in the single micron size range which can make it difficult to analyse for polymer composition (e.g., Alurralde *et al*. 2022; Frias *et al*. 2010).

As the chemical analysis is time consuming, costly and may be access-limited and in the absence of automated technology can require researchers to handpick suspected microplastics to be manually analysed by Raman or FTIR spectroscopy a subsample is selected from suspected microplastics for analysis (Nie *et al*. 2019; Gündoğdu *et al*. 2020). From the literature a variety of subsamples are taken for chemical analysis. Ziajahromi *et al*. (2017) analysed 10% of suspected particles. Covernton *et al*. (2019) analysed 7/338 suspected particles from oysters, 9/253 from clams, 10/295 from sediment and 18/289 found in water samples. Baechler *et al*. (2020) analysed 26 of 3053 suspected microplastics found in bivalves. Li *et al*. (2016) analysed 129 of 1519 suspected particles extracted from mussels. Kazour and Amara, (2021) examined all sediment items and a subsample of water items using Raman spectroscopy from the French coastline. Mani *et al*. (2015) analysed 118 of 25956 suspected particles recovered from water samples from the River Rhine which was designed to cover putative plastic particles from a) every sampling location as well as b) from every category found (fragments, fibres, spherules, etc.). Eighty-seven micro particles collected from surface water from the Three Gorges Reservoir, China were analysed, however, the % of the total microplastics recovered was not mentioned (Di and Wang, 2018). Su *et al*. (2016) analysed

113 particles or 6% of the total recovered from surface water and sediment from Taihu Lake in China, while O'Connor *et al*. (2020) examined 30 microplastics recovered from the gastrointestinal tract and stomach contents of the brown trout, *Salmo trutta*, from the River Slaney catchment in Ireland. Further evidence of the subjectivity of chemical analysis was shown in Piarulli *et al*. (2019) where two separate scientists studied all filters and where there was doubt over a particle being synthetic or natural it was selected for analysis by FTIR.

2.9.1 Raman Spectroscopy for microplastics identification

Raman spectroscopy is one of several identification methods used to determine the polymer of microplastic under study. It is a vibrational spectroscopy technique based on the inelastic light scattering providing information on the molecular vibrations of a system in the form a vibrational spectrum. The spectrum generated is similar to the fingerprint of chemical structure thus enabling identification of the components in a sample (Araujo *et al*. 2018). Raman spectroscopy has been used to identify microplastic polymers extracted from wastewater (e.g., Lares *et al*. 2018), seawater (e.g., Karlsson *et al*. 2017), freshwater (e.g., Di and Wang, 2018), sediment (e.g., Lots *et al*. 2017) and aquatic organisms (e.g., Horton *et al*. 2018). In common with FTIR, Raman techniques are in general non-destructive, only require a small amount of sample, have the potential for high throughput screening and are environmentally friendly. Raman spectroscopy has better spatial resolution when compared to FTIR techniques as well as wider spectral coverage, lower water interferences and narrow spectral bands (Araujo *et al*. 2018). There are some drawbacks to using Raman spectroscopic analysis one being that additives such as dyes or plasticizers can cause difficulties interpreting Raman signatures (Nava *et al*. 2021). Raman spectroscopy additionally has an inherently low signal to noise ratio and may cause sample heating due to the use of a laser as a light source which can result in polymer degradation and sample loss (Araujo *et al*. 2018). Additionally, the Raman spectra generated are simple / specific so that the preparation of samples can be simplified (Fang *et al*. 2023; Madejová, 2003). However, the high price of the equipment is a noted drawback (Fang *et al*. 2023; Zada *et al*. 2018). A number of factors can hinder Raman analysis such as; signal saturation due to high fluorescence (particularly true for bright microplastics), mismatched results from databases or the absence of clear peaks in spectra hindered microplastic identification. Additionally, Raman signals have difficulty recognising chemical compounds of dark-coloured particles due to melting or burning when the energy of the laser is absorbed by the sample (Young and Elliot, 2016). Similar to the analysis carried out by Khuyen *et al*.

(2021) particles that were homogenous in shape and possessed clear colour were classed as unidentified microplastics as they were likely anthropogenic in nature.

2.9.2 Difficulties in comparing results

The real-world difficulties associated with chemical identification of suspected microplastics were encountered in the work carried out in chapters 3 – 5 of this thesis. The following spectra / one similar to this was encountered in several environmental matrices studied as part of this thesis (Fig. 2-3). Using the in-house libraries (SLoPP and SLoPP-E) several possible matches for polymers (e.g., PA, PE and Polyethylene-carbonate) were obtained. While the best match using the online and commonly used libraries of OpenSpecy and PublicSpectra similarly matched over 70% with PA and several other polymers primarily based on the peaks just below 3000 cm^{-1} (Fig. 2-4) which is commonly associated with synthetic polymers, however the region between 1000 cm^{-1} and 1500 cm^{-1} peaks were much greater (Fig 2-5). It was postulated that this may be due to some additives / dyes altering the spectra in this region, nevertheless, several researchers who had recently published environmental microplastics research papers using Raman as an identification tool were reached out to for their opinions on what it may be.

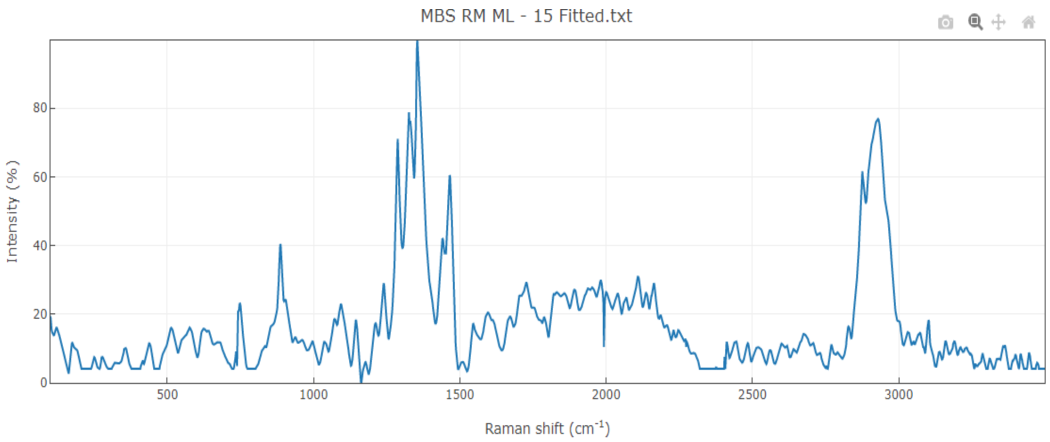
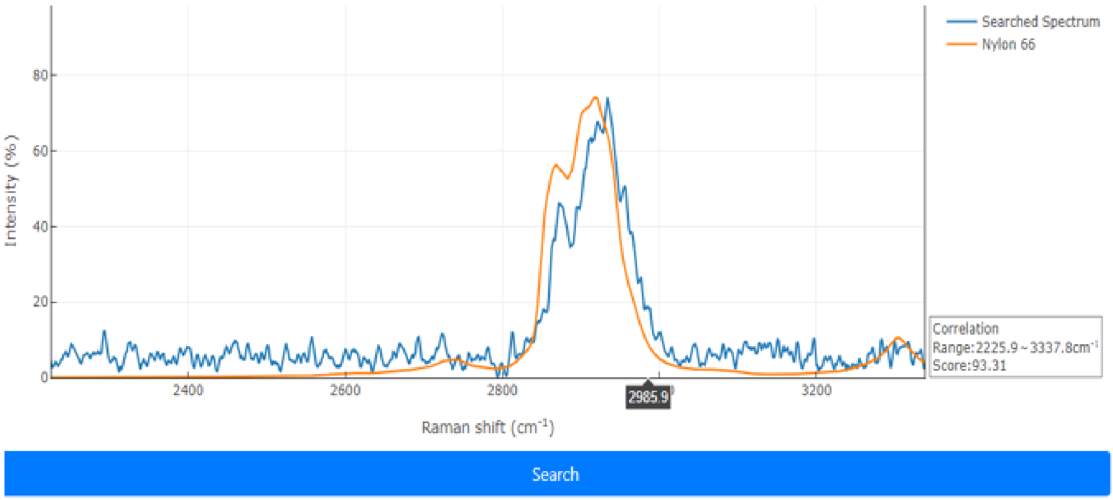


Figure 2-3: Raman spectrum of polymer that was sent to other researchers to examine.



#	Score	Material Name	Chemical formula
1	96.37	Nylon 46	(C10H18N2O2)n
2	93.89	Ethylene tetracyclododecene copolymer	(C2H4)n (C12H16)m
3	93.87	Polyisoprene, CIS	(C5H8)n
4	93.31	Nylon 66	(C12H22N2O2)n

Figure 2-4: Raman spectrum showing region around 3000cm^{-1} matching to PA of unspecified polymer using PublicSpectra (https://publicspectra.com/SpectralSearch).

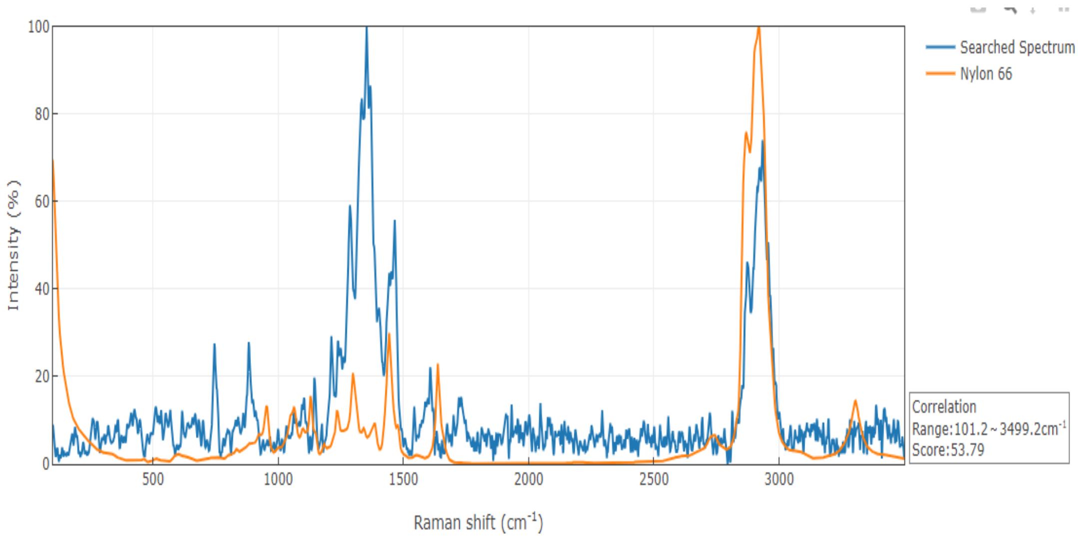


Figure 2-5: Raman spectrum displaying best match using PublicSpectra online library for this polymer (https://publicspectra.com/SpectralSearch).

One researcher suggested that the large peaks displaying were due to fluorescence that was skewing the spectrum generated and to leave the laser on the sample for longer to try and reduce this effect. This was trialled but did not change the spectra generated. Another researcher postulated that the fact that this spectrum was appearing in multiple different environments possibly indicated that the microplastics were acting as a sort of environmental indicator for a pollutant that was present and adsorbing to its surface especially if the microplastics were of different shapes and colours. Alternatively, they suggested that the polymer was polyamide and that there was interference from something on the surface (natural organic matter) or a pigment in the plastic. A creator of one of the online spectra libraries gave their opinion following analysis of the spectra through their software. They stated that they did not get great matches and the best was a 70% match to a pharmaceutical used as an emulsifier of natural origin. When queried with the hypothesis that it could be a polyamide with a dye or additive included, they said that that was just as likely as their suggestion stating that amides and organic matter are notoriously difficult to differentiate from each other. The polymer was also not noted in any blanks that were analysed using Raman. The diversity of answers amongst researchers that had used Raman for microplastic identification underlines the difficulty in comparing identified polymers between studies. Where encountered this spectrum and those similar was classed as an “unspecified polymer” as it displayed Raman characteristics with other polymers but it could not be definitely classed as one particular polymer.

2.10 Conclusion

As microplastic research is inundated with a diverse range of novel methodological approaches and publications utilising different processing and isolation steps (Lusher *et al*. 2020) there are numerous methods available to choose from for researchers which can be tailored for their specific research goals. Additionally, studies on environmental science should inherently aim to be environmentally friendly in their approach (Mani *et al*. 2019). In summary, it is likely that no microplastics sampling technique will garner 100% accurate measurements from environmental samples but by selecting an appropriate methodology coupled with adequate controls and chemical composition analysis a reliable set of results can be produced. The difficulties in achieving standardisation in microplastics sampling is highlighted in the work presented in the following thesis. While castor oil achieved good separation for marine sediment sampled in chapter 4 it was not viable for use in chapter 3 as stones present in the riverine sediment blocked the stopcock in the filtration unit and potassium carbonate in beakers were used instead, however both methods had comparable safety, cost and efficiency in extracting microplastics from their respective substrate.

Chapter 3: Comparative assessment of microplastics in sediment, surface water and *Gammarus duebeni* of Irish rivers

Stephen Kneel*, Caroline Gilleran Stephe ns[1], Alec Rolston[2], Niamh Tester[1], Ana Marques Mendes[3], Liam Morrison[3] Suzanne Linnane[1]

[1]Centre for Freshwater and Environmental Studies, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland

[2]Goyder Institute for Water Research, Adelaide, South Australia, Australia.

[3]Earth and Ocean Sciences, School of Natural Sciences and Ryan Institute, National University of Ireland Galway, Ireland

*Corresponding author: stephen.kneel@dkit.ie

3.1 Abstract

Currently microplastics research in Irish freshwater environments is limited but growing with a number of studies published in recent years (e.g., Barrow *et al*. 2022; O'Connor *et al*. 2020). This study assessed the rivers of a previously unstudied freshwater catchment for microplastic pollution on the East Coast of Ireland. Microplastics were characterised from surface water samples for two sites (headwater and outflow) on seven rivers (n = 14) for two sampling campaigns (2019 and 2020-21) (n = 28 total site visits). Samples collected from the first campaign (mean = 1.6 ± 2.24 MPs L^{-1}, median 0.81 MPs L^{-1}) had a significantly higher concentration than those collected in the second sampling campaign (mean = 1.14 ± 1.74 MPs L^{-1}, median = 0.45 MPs L^{-1}) and may be attributed to the increased rainfall observed during the first sampling campaign. Additionally, one river, which displayed large differences in microplastic concentrations in surface water samples between its headwater and outflow sites across both sampling campagns, was sampled for *Gammarus duebeni* in 2022 together with riverbank sediment to determine if this difference was reflected in other freshwater parameters. There were significant differences in microplastic concentrations in sediment between the headwater (180 MPs kg^{-1}) and outflow (370 MPs kg^{-1}) study sites but this was not the case for *G. duebeni* samples (0.059 MPs $mgtissue^{-1}$ and 0.052 MPs $mgtissue^{-1}$). Microplastics were found in every sample taken and environmental matrix examined (water, sediment, *G. duebeni*). Finally, we found higher microplastic concentrations were present in surface water taken at both the urban sampling site and one rural site but we attributed elevated microplastic concentrations to different causes; population pressure and land-slope respectively. This study adds to the growing body of knowledge of freshwater microplastic pollution and examines the

levels of microplastics in rivers that flow into the Special Conservation Area (SAC) and Special Protected Area (SPA) of Dundalk Bay which is unexamined in terms of this pollutant.

Graphical abstract

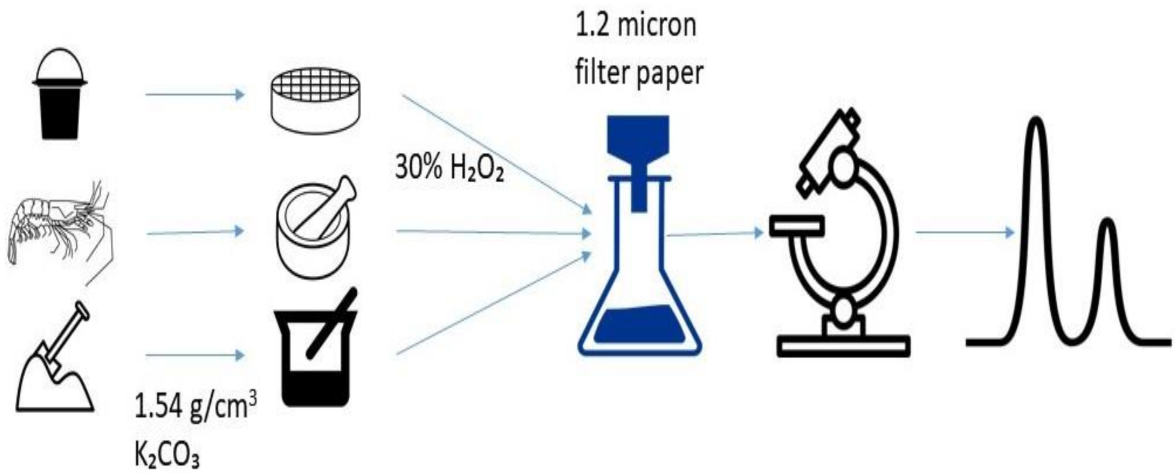


Figure 3-1: Graphical abstract showing workflow from sample collection to Raman Spectroscopic analysis.

Keywords

Microplastics, rivers, sediment, freshwater pollution, macroinvertebrates

3.2 Introduction

Existing in two categories; primary and secondary, microplastics have become a contaminant of emerging environmental concern and are defined as "*any synthetic solid particle or polymeric matrix, with regular or irregular shape and with size ranging from 1 µm to 5 mm, of either primary or secondary manufacturing origin, which are insoluble in water*" (Frias and Nash, 2019). To date the majority of papers written on the subject of microplastics are primarily focused on marine ecosystems, for example a literature review conducted by Cera *et al*. (2020) found that from 2012 to 2020, 2864 papers were published on microplastics and marine ecosystems while just 158 were based on freshwater ecosystems. While there has been an increase in studies on freshwater microplastic pollution in recent years there has been relatively little work carried out on Irish freshwater environments. Sediment in the River Barrow system has been shown to be contaminated with microplastics (Murphy *et al*. 2022), microplastics have been documented in the stomach contents and gastro-intestinal tract of brown trout (Salmo trutta) (O'Connor *et al*.0 2020) while a preliminary study detected microplastics in river, lake and wastewater from several Irish locations (Cedro and Cleary, 2015). Microplastics have also been documented in the human body with some pigmented particles found in the placenta (Ragusa *et al*. 2021).

Rivers act as important transporters of plastic waste from the terrestrial environment to the marine one with the quantities transported expected to increase in the future (Jambeck *et al*. 2015). It is estimated that 1.15 to 2.41 million tonnes of plastic enter the ocean via river transport every year (Lebreton *et al*. 2017). Furthermore, around 80% of marine microplastics enter the ocean through riverine transport (Mani *et al*. 2015). Modelling work on Europe's largest rivers found that the majority of microplastics exported by rivers to seas are from tyre and road wear particles (42%) and fibres (polyester etc., 29%) which are shed from items of clothing during washing (Siegfried *et al*. 2017). Other sources of microplastics to freshwater environments include; storm water run-off which can dump microplastics from land-based activities into freshwater systems (Cho *et al*. 2023) and waste-water treatment plants (Conley *et al*. 2019). For example, the mass release of tyre and road wear particles was estimated for the Seine watershed at $1.8\text{kg inhabitant}^{-1}\text{ yr}^{-1}$ (Unice *et al*. 2019). As an anthropogenic pollutant it is therefore unsurprising that urban development close to or on freshwater rivers leads to a high abundance of microplastics in these rivers and their sediment (Kunz *et al*. 2023; Peng *et al*. 2018). Not only can rivers serve as transporters of terrestrial plastic to the marine environment but they can also hold them as temporary sinks. A study tracking the movement of GPS-tagged bottles in the Seine observed that 100% of bottles were deposited on riverbanks for hours to weeks with only one bottle making it to the ocean (Tramoy *et al*. 2020). The fact that the tracked bottles were always found with other plastic types indicates that rivers can hold macroplastic debris for long periods of time resulting in secondary microplastic formation in the environment with the authors noting that cleaning activities should focus on riverbanks and not on the open ocean (Tramoy *et al*. 2020).

Anthropogenic pressure is noted to increase the abundance of microplastics in waterways as well as their diversity of types (Govender *et al*. 2020). Microplastics that end up in freshwater systems can have various terrestrial origins. Several studies have evaluated the presence of microplastics in wastewater treatment plant (WWTP) effluent with varying results. It has been reported that WWTPs remove approximately between 90 and 95% of microplastics from their influent (Leslie *et al*. 2012; Talvitie and Heinonen, 2014). However, another study has shown that microplastic removal from raw wastewater can be as high as 97% where fibres were less likely to be captured during treatment accounting for only 74% of total microplastics in raw wastewater but accounting for 91% of microplastics in treated effluent (Ben-David *et al*. 2021). In countries such as Ireland where sludge from WWTP is used for agricultural applications as biosolids, microplastics trapped during treatment of wastewater in sludge form may end up re-entering the freshwater environment if they are washed from fields into river bodies or lakes due to precipitation. Approximately 98% of biosolids produced in Ireland are reused on agricultural land and by 2040 sewage sludge production is expected to increase by 80% (Uisce Éireann, 2023).

Recorded microplastic pollution levels in freshwater systems differ greatly between studies (e.g. Bordo *et al*. 2021; Rowley *et al.* 2020). These differences may be due in part to differing waste management systems between countries and locations of rivers (with regard to possible microplastic emission sources). In European rivers there exists large variances in microplastic exports due to differences in socio-economic development among countries and regions and the technological status of sewage treatment facilities (Siegfried *et al*. 2017). Microplastics unlike chemicals which dissolve in the water body, exhibit variation within the water column with regards to distribution due to their inherent properties and interactions with surrounding substances (Kye *et al*. 2023). Some physical factors related to the water body which may influence microplastic distribution in rivers include; riverbed morphology, water column confluence depth and obstacles in the river as well as variations in river discharge (Best 1988; Walling and Moorehead 1987). Turbulent conditions and fast flow in the main river body can lead to increased mixing of particles in the water column due to resuspension and transport (Haberstroh *et al*. 2021; Hurley *et al*. 2018; Ockelford *et al*. 2020). On the other hand, low-energy zones such as along river banks, bends in the river or dams can facilitate the deposition of microplastics in sediment (Corcoran *et al*. 2020; Crew *et al*. 2020; Watkins *et al*. 2019). In marine environs, surface sediment in the shore zone could reflect long-term interaction between the aquatic and terrestrial environment and historic microplastic presence (Yu *et al*. 2016).

Microplastics are found in all freshwater systems and display a top-down distribution gradient (Bellasi *et al*. 2020). In surface waters, the water column and in sediments, the density of plastic affects the partitioning of material such as organic matter or contaminants (Li *et al*. 2018). Transport of microplastics in freshwater systems depend on hydrodynamic properties such as size, density, shape, surface roughness and also conditions of the open channel flow of the river (Frei *et al*. 2019). Lower density plastics which are expected to float have been noted to sink in freshwater bodies due to the occurrence of biofouling by algae and bacteria among other species (Corcoran 2015). In addition to sinking due to biofouling, lower density plastics can also sink due to the process of aggregation which can occur with other microplastics or with organic material present in the water column (Leiser *et al*. 2021) as well as being egested as part of a faecal pellet (Cole *et al*. 2016). Microplastics may then enter the hyporheic zone potentially interacting with organisms found there or entering the groundwater system beneath a river (Frei *et al*. 2019).

Microplastic concentrations have been shown to span several orders of magnitude in different locations (Xu *et al*. 2020). An example of the huge range of microplastics concentration recorded in surface waters is the Tamar estuary in England which possessed 0.028 particles/m^3 (Sadri and Thompson 2014) and the Saigon River in Vietnam where 172,000-419,000 microplastic items/m^3 were found (Lahens *et al*. 2018). Freshwater sediments have also been studied as a major sink of microplastics, effectively trapping microplastic particles before they reach estuarine or marine environments. A study on the Yangtze River Delta's surface water and sediment found a larger number of microplastics in the sediment than in the surface water, 0.48-21.52 items L^{-1} and 35.76-3185.33 items kg^{-1} dry weight respectively (Hu *et al*. 2018). In general, however, the comparison of data between studies is difficult and standardisation of sampling and separation methods is lacking. Differences in reported concentrations may therefore be due to methodologies used rather than reflecting true environmental abundances.

The majority of studies on microplastics in biota have focused on marine species with a comparatively lower number assessing microplastic contamination amongst freshwater species. Freshwater vertebrates such as fish and birds have been investigated for microplastic contamination more than microorganisms or invertebrates (Cera and Scalici, 2021). Amongst freshwater invertebrates, bivalves are the main group that is studied in freshwater systems (Cera and Scalici 2021). While bivalves have potential as bioindicators for microplastic pollution of aquatic ecosystems (Ding *et al*. 2021, Staichak *et al*. 2021) the lack of diversity in research represents an oversight in microplastic freshwater study. Arthropa (insects and crustaceans) are a diverse group of species representing different feeding mechanisms and existing at different levels on food webs and therefore can be exposed to varying levels of microplastics and pose as an entry point for these contaminants enter freshwater food webs. Members of the arthropod family have been shown to consume microplastics in their natural environment as well incorporate them into the building of their protective casings (Nel *et al*. 2018; Windsor *et al*. 2019; Simmerman and Coleman Wasik, 2020; Ehlers *et al*. 2019). The varying sensitivities of certain macroinvertebrates groups to pollutants is well documented and in Ireland the Environmental Protection Agency (EPA) has used the Q-value scale (1 (poor quality) – 5 (high quality)) in Irish rivers since the 1970's to assess water quality based on the well-established sensitivities, abundance and diversity of macroinvertebrates. As primary consumers freshwater macroinvertebrates may represent starting points of microplastics entering the food web as they are prey for higher species such as a fish, birds, amphibians and reptiles, therefore, it is important to understand the uptake levels of microplastics to this group of species in their natural environments. Additionally, fish larvae and plankton which serve as an important food source for many freshwater species can be outnumbered by floating microplastics, at ratios up to ∼30:1 and can exist in a similar size range in the water column (Lechner *et al*. 2014; Steer *et al*. 2017).

Currently, there still exists a lack of robust evidence that quantifies links from sources and the fate of microplastics in freshwater bodies and the marine environment with less attention given to smaller order rivers or streams (Dikareva and Simon, 2019). As a country that uses wastewater treatment plant sludge as biosolids for agricultural applications, the freshwater environments of Ireland may be particularly at risk for microplastic pollution (Rolsky *et al*. 2020). Only low numbers of freshwater studies on microplastic pollution in Ireland are currently available (e.g., Murphy *et al*. 2022; O'Connor *et al*. 2022). It is of paramount importance to increase the number of studies carried out to better understand the levels of microplastic contamination in freshwater environments and potential threats posed by them. Therefore, in order to understand the levels of microplastics present in rivers flowing into the marine environment of Dundalk Bay (Special Area of Conservation and Special Protected Area) the surface water, sediment and the macroinvertebrate species, *Gammarus duebeni*, were examined.

With these factors in mind the overall aim of this study was to determine the extent of microplastic contamination in river systems flowing into Dundalk Bay. The objectives of this study were threefold: 1) To characterise and quantify the levels of microplastics present in river surface water across two sampling campaigns and note any differences between sampling campaigns and locations of sites and explain these differences. 2) To characterise and quantify the levels of microplastics in riverbank sediment and *G. duebeni* in selected water bodies. 3) To examine how microplastics characteristics reflect each other between the three matrices studied.

3.3 Material and methods

3.3.1 Study site

In this study, seven rivers in the North-East of Leinster that flow into Dundalk Bay through the county of Louth on the east coast of Ireland were examined for the presence of microplastics. Although the smallest in terms of geographical area (approx.: 825km^2), the county of Louth possesses the second highest population density in the country of Ireland at 155.9 inhabitants km^{-2} more than double the national average featuring urban centres and rural areas. Given this, Louth is an ideal location to study microplastic presence in rivers that have both high and low population pressures (<20 - >1000 inhabitants km^{-2}) and that flow into the economically and environmentally important marine bay in Dundalk. Dundalk Bay is a large open shallow sea bay and the extensive sand and mud flats have a rich fauna of bivalves, molluscs, marine worms and crustaceans that help to support the large colonies of waterfowl that reside there in winter months (Dundalk Bay SPA: 004026). Additionally, Dundalk Bay hosts a heavily regulated cockle fishery that generated €80,500 average value per vessel for 2020 (Fisheries Natura Plan for cockle 2016-2020). The rivers investigated during this study, the Ramparts, Dee, Flurry, Big, Glyde, Castletown and the Fane, flow through land with various uses. Peat bogs, coniferous and mixed forests and industrial areas are present, however, the area inland is dominated by agriculture while the town of Dundalk and its suburb Blackrock dominate the coastline as urban areas (Corine Land Cover, 2018 European Environment Agency). The largest urban centre is Dundalk. The total population of the catchment (Republic of Ireland) is approximately 115,900, with a population density of 83 people per km^2 (3rd Cycle Draft: Newry, Fane, Glyde and Dee Catchment Report, EPA, 2021).

Water sampling was conducted over the course of two regimes; Sampling for the first campaign was carried out in September and October of 2019 while for the second campaign it was carried out between 29th September 2020 and November of 2020 with one site sampled in January of 2021 (due to Covid-19 related disruptions). Two points along seven rivers were studied along the river's course for surface water microplastic abundance. Sediment and *G. duebeni* collection was carried out on the 25th of February 2022 from the river Flurry at both headwater and outflow locations. In total, 14 sites were selected for water sampling (2 per river), one upstream (headwater) location which was further inland than the other sampling site located further downstream (outflow) (Fig. 3-2; Table. 3-1, 3-2). Sites were selected based on ease of access and near to their emergence points for headwater locations while for outflow sites this was at locations before the rivers entered the bay. A map displaying the Q-values for the rivers sampled is located in Appendix A. (Fig. 8-1).

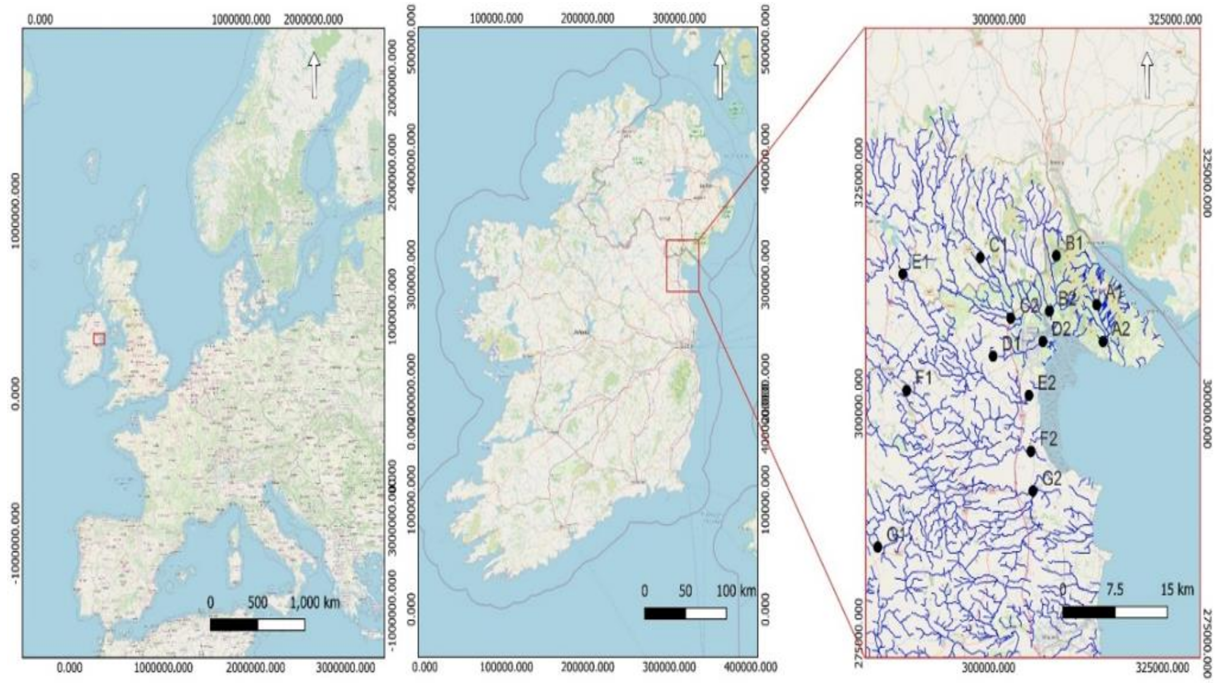


Figure 3-2: Location of sampling sites on rivers flowing into Dundalk Bay.

Table 3-1: Site Codes and Descriptors.

Site Code	Site Name	Q Value*	Coordinates	Catchment km^2
A1	River Big Headwater	4, Good	54.003511, -6.2283468	7.7
A2	River Big Outflow	3-4, Moderate	54.039427, -6.2421159	22.3
B1	River Flurry Headwater	3, Poor	54.087064, -6.3298153	Not available
B2	River Flurry Outflow	3 – 4, Moderate	54.033148, -6.3452434	24.1
C1	Castletown River Headwater	4, Good	54.085142, -6.495563	Not available
C2	Castletown River Outflow	3 – 4, Moderate	54.026291, -6.4292035	33.8
D1	Ramparts River Headwater	No Q value available	53.989324, -6.4676632	7.2
D2	Ramparts River Outflow	No Q value available	54.003594, -6.3591319	22.2
E1	River Fane Headwater	3, Poor	54.069255, -6.6642361	Not available
E2	River Fane Outflow	4, Good	53.951103, -6.3895841	275.2
F1	River Glyde Headwater	3 – 4, Moderate	53.95584, -6.6558974	87.9
F2	River Glyde Outflow	3 – 4, Moderate	53.896488, -6.3852067	359.9
G1	River Dee Headwater	3, Poor	53.802958, -6.7188338	108.3
G2	River Dee Outflow	4, Good	53.857813, -6.380868	317.7

*all data taken from 2020 EPA reports, the values included are from the closest and most recent possible location to sampling locations that is available.

Table 3-2: Flow rate data for rivers investigated. (Environmental Protection Agency, https://gis.epa.ie/EPAMaps/Water).

River	Mean Flow rates (m^3/s)	Min flow rate (m^3/s)	Max Flow rate (m^3/s)
Big	0.54	0.04	3.22
Flurry	0.71	0.05	3.92
Castletown	0.1	0.01	0.58
Ramparts	0.36	0.03	2.01
Fane	4.85	0.42	21.1
Glyde	5.82	0.39	25.28
Dee	5.72	0.35	28.11

3.3.2 Study organism: *Gammarus duebeni*

Not only do freshwater systems serve as transporters of microplastics to marine environments they can also function as sinks for microplastic pollution. Therefore, it is important to examine microplastic contamination of freshwater environmental matrices and biota that reside in these areas. The freshwater amphipod, *Gammarus duebeni*, was chosen to assess microplastic contamination in the River Flurry as part of this study. *G. duebeni* are leaf-shredding crustaceans found in Irish freshwater systems. They feed on organic detritus and are themselves prey for freshwater fish. Although they are a benthic species, they can swim in the water column and feed on floating plant material, this in turn means they are potentially susceptible to microplastic contamination found in surface water of rivers and lakes and in the sediment of them. Additionally, *G. duebeni* has been used in Irish lab-based microplastic studies in the past and shown to consume microplastics and shred them to smaller sizes (Mateos-Cárdenas *et al*. 2020, 2019). Given its tolerance for a wide-range of salinity *G. duebeni* has potential as both a biomonitor of microplastic pollution of freshwater and marine environments, furthermore, *Gammarus* sp. are considered model ecotoxicological freshwater species (Consolandi *et al*. 2019). To the best of the author's knowledge this study represents the first investigation into microplastic ingestion by *G. duebeni* in the natural environment.

3.3.3 Sample collection

A bulk water sampling procedure was carried out as follows: At each sampling site 200 litres of surface water was collected using a pink brightly coloured plastic bucket with the exception of the outflow sites of the Rivers Ramparts and Flurry where 20 litres were collected respectively. A pilot study revealed microplastic concentrations too numerous to count for these two sites so a smaller volume was taken. Prior to sampling the bucket was rinsed out using river water. Following this, the bucket was pushed down into the water, open side up, allowing it to fill with river water. Surface water at each site was passed through a series of 5 interlocked stainless-steel sieves of decreasing mesh size (5mm, 1mm, 500µm, 100µm and 50µm). The use of buckets for surface water sampling has also been used by; Suresh *et al*. (2020), Miller *et al*. (2017) and more recently Osorio *et al*. (2021). The use of buckets to collect a bulk sample of water ensure that smaller microplastics generally <300µm that are missed by more commonly used nets are retained for analysis. Following filtration, sieves were covered in tinfoil and transported to the microplastic clean room for further analysis. Both sieves and buckets were triple rinsed with Milli-Q water prior to use in sample collection and covered in tinfoil for transport to site.

In February 2022, sediment and macroinvertebrate samples were collected from the outflow and headwater of the River Flurry. The River Flurry was chosen to examine microplastic presence in riverbank and *G. duebeni* as this river displayed a large difference in surface water microplastics for the two sampling locations for both sampling campaigns while also hosting a *G. duebeni* population and safe access to the riverbanks. A metal trowel was used to collect samples of sediment (1–5cm) from the riverbank and these were stored in aluminium containers and covered in foil before being transported to the lab and oven dried at 40°C overnight. Freshwater macroinvertebrates were collected from the riverbed using 2-minute kick samples from the faster flowing riffle habitats in triplicate. Freshwater macroinvertebrates were stored in plastic bags for transport to the laboratory. The duration of time between sampling and storage of *G. duebeni* was approximately 20 minutes.

3.3.4 Processing of water samples

In the laboratory, sieves were cleaned of any material which had been caught on them through a combination of scraping using a metal spatula and rinsing the sieve using Milli-Q (0.22µm) water. All of the material removed from the sieves was stored in a glass jar (acid-washed, 1% nitric acid and triple rinsed with Milli-Q water) with a metal lid. This was carried out for each individual sieve with the exception of the 5mm sieve, in this instance any material caught was discarded as 5mm is generally accepted as the upper size limit of microplastics (Anderson *et al*. 2016; Frias and Nash, 2019). Samples were stored in a cold room and further sample treatment was carried out as soon as possible following sieve cleaning.

Centrifugation in combination with organic material digestion with 30% Hydrogen peroxide (H_2O_2) was carried out (Di and Wang 2018; Liebezeit and Dubaish, 2012). Briefly, samples were centrifuged at 3500rpm in glass centrifuge tubes for 5 minutes (Canensi *et al*. 2022; Van Cauwenberghe *et al*. 2013). The supernatant was then vacuum filtered through glass microfibre filter papers (GF/C; 1.2µm; 47mm in diameter, Whatman, UK). The walls of the centrifuge tube and vacuum filtration headpiece were rinsed with Milli-Q water to remove any attached microplastics. Following supernatant vacuum-filtration, filter papers were placed in petri dishes, covered and stored in a desiccator prior to visual inspection. The organic material remaining in the centrifuge tubes following supernatant filtration was treated with 10ml aliquots of 30% H_2O_2 which were then covered in tinfoil and digested at 40ºC for 48 hours. Vacuum-filtration of the digested pellets was carried out identically to the supernatant and filter papers were stored in petri dishes in a desiccator for 24 hours prior to visual analysis.

3.3.5 Processing of sediment samples

Microplastics were extracted from river sediment using density separation. A protocol was adapted from Gohla *et al*. (2020) for this extraction. Briefly, four replicate 50g samples of sediment were placed in 500ml beakers to which 200ml of potassium carbonate (K_2CO_3) with a density of 1.54 g/cm^3 was added. Samples were mixed using a magnetic stirrer for 2 minutes, covered with tinfoil and left for one hour to settle. Following this, the supernatant was vacuum filtered (Whatman 1822-047 GF/C Glass Microfiber Filters, 1.2um, 4.7cm). The procedure was repeated in order to obtain maximum possible extraction.

3.3.6 Processing of *G. duebeni* samples

Upon returning to the lab *G. duebeni* were stored in glass jars with Industrial Methylated Spirits (IMS) and samples were then stored in a cold room prior to subsequent work. The methodology was similar to that detailed in Avio *et al*. (2015). Preserved samples were emptied onto shallow trays for *G. duebeni* extraction using steel forceps. Individual organisms (n = 80) were carefully removed from the tray and rinsed using Milli-Q water in order to remove any potential microplastics stuck to the exterior of the organisms. Groups of 5 *G. duebeni* were selected to form composite samples, weighed and then homogenised using a mortar and pestle. The homogenised samples were poured into glass petri dishes for digestion, the mortar and pestle were triple rinsed using Milli-Q water between homogenisations to avoid cross contamination of potential microplastics between groups. Composite samples were digested with a 20 ml volume of 30% H_2O_2 for a 24-hour digestion at 40ºC. Following the 24-hour digestion period, digested samples were vacuum filtered onto filter papers (Whatman 1822-047 GF/C Glass Microfiber Filters, 1.2um, 4.7cm) and placed in labelled petri dishes. Petri dishes which had been used for *G. duebeni* digestions were rinsed using Milli-Q water to ensure no material was left behind.

3.3.7 Quality control measures

It is of the utmost importance to ensure good laboratory practises when analysing samples for microplastics. Protocols for mitigating microplastic contamination were adhered to where applicable from Hermsen *et al*. (2018). As microplastics can shed from clothing, 100% cotton lab coats were worn when conducting analysis or handling samples. Milli-Q (0.22 µm) water was used for the rinsing of sieves and other lab work. Vacuum filtering of water samples, *G. duebeni* homogenisation and sediment density separations were all carried out under a laminar flow hood. All work conducted on samples including the aforementioned vacuum-filtration and sample handling was carried out in a "clean room" which was used exclusively for microplastic work. When sample processing was carried out, a wet filter paper was left out on in the laminar flow hood in a petri dish for the same duration and visually examined under the microscope for any microplastics that may have settled on it. Additionally, blank solutions were also processed in conjunction for samples. The results of these controls are displayed in Appendix A (Tables; 8-2, 8-3, 8-4, 8-5) and were considered negligible when compared to the amount of microplastics recovered from each sample matrix thus no correction measurement was carried out. Equipment such as; tweezers, sieves and glassware were covered in tinfoil when not in use. Only one individual was allowed in the clean room while sample processing or microscopic work (also located within clean room) was carried out.

To avoid contamination of airborne microplastics sieves were transported to and from river sites covered in foil. Buckets and glassware used for sample collection and analysis were triple-rinsed using Milli-Q water before use in the field. These measures were necessary to avoid microplastic contamination from particles that may be present in the air and settle on equipment leading to an overestimation of microplastic numbers. Fibrous microplastics which are present in outdoor and indoor air have the potential to settle on equipment which may lead to an overestimation of the amount present in a sample matrix (Gasperi *et al*. 2018).

3.3.8 Microplastic identification

All filters were sorted by visual inspection using an Olympus SZX7 stereomicroscope and microplastics were noted, measured in size, described and photographed. Following Gewart *et al*. (2017), metal tweezers were used to check if suspected particles were microplastics based on their texture. The structural integrity when touched or moved by tweezers was used to identify suspected microplastics, avoiding those with biological structures (Hurley *et al*. 2018). Organic and inorganic material such as algae or sands tend to break or crumble when pressure is applied using tweezers however microplastics resist this pressure and are stiffer in nature (Keene and Turner 2023). This was particularly important when testing the nature of the carapaces of *G. duebeni* which can resemble transparent films visually. Microplastics were categorised in terms of morphology as one of; fibres, films, fragments, microbeads or as a fibre agglomeration. Microplastic colour was also noted and red, blue, green, transparent/white, and black microplastics were recorded while less common colours were classed as "other".

3.3.9 Raman identification of polymers

A subsample of particles from surface water, *G. duebeni* and sediment samples collected were analysed for polymer identification using Raman spectroscopy (Nie *et al*. 2019; Gündoğdu *et al*. 2020). Display slides with double sided sticky tape attached were used to house microplastics for Raman analysis. Using tweezers suspected microplastics were transferred from filter papers to the slides under the microscope and a small circle was drawn around the now attached particle in order to enable quick location when analysing with Raman spectroscopy (Horiba LabRAM II, Horiba Jobin-Yvon, France). The Raman Spectrometer was equipped with a 600 groove mm^{-1} diffraction grating, a confocal optical system, a Peltier-cooled Charged couple device (CCD) detector, and an Olympus BX41 microscope (Ó Briain *et al*. 2020; Loughlin *et al*. 2021) and spectra were obtained at a range of 100–3500 cm^{-1} using a 532 nm laser for polymer identification. Spectra obtained when analysing particles extracted from *G. duebeni*, water and sediment were compared to a spectral reference library (KnowItAll, Bio-Rad), an in-house extension of the library with additional spectra from environmental plastics collected from the intertidal zone and known virgin polymer types (purchased from CARAT GmbH, Bocholt, Germany). In addition, SLoPP and SLoPP-E libraries (Munno *et al.* 2020). The website 'Open Specy' (Cowger *et al*. 2021) was also used to verify polymer type for spectra captured via Raman (https://openanalysis.org/openspecy/) as well as the website 'PublicSpectra' (https://publicspectra.com/). Furthermore, the Infrared & Raman Users Group (http://www.irug.org/search-spectral-database) was also consulted.

3.3.10 Statistical analysis

Due to the different methods used for sampling water, sediments and *G. duebeni* the data were analysed separately. Data from sediment samples, water samples and *G. duebeni* samples were tested for normality using the Ryan-Joiner normality test. Two sample t-tests were used to assess if any statistically significant differences were present in *G. duebeni* samples between the two sample sites on the River Flurry both in terms of MPs $individual^{-1}$ and MPs mg^{-1} and also to determine if concentration differences in sediment were significant. As data from the surface water samples were deemed to not be normally distributed Mann-Whitney testing was carried out to determine if the median concentrations of the two sampling campaigns were significantly different, and if the median concentrations between the combined sampling campaigns differed for headwater and outflow sites and for individual years. Kruskall-Wallis testing was carried out for both sampling campaigns to determine if any locations had significantly difference microplastic levels. Significance was determined for all tests as $P < 0.05$. Statistical analysis was carried out using Microsoft Excel 2016 and Minitab Statistical Software version Minitab® 21.1.1 (64-bit)) with the latter used for graph building also.

3.4 Results

3.4.1 Microplastics in river water

Microplastics were found in all samples, recovered from every site and from both sampling regimes for surface water of rivers that were assessed during this study with a variety of shapes found (Fig 3-3). The total volume of water sampled was approximately 2440 litres for each sampling campaign. Microplastic concentrations ranged from 0.45 MP L^{-1} to 8.55 MP L^{-1} for surface water sampled during the first campaign and from 0.23 MP L^{-1} to 5.30 MP L^{-1} for the second campaign. Levels of microplastics found in surface water decreased at 11 sampling sites between campaigns while there was an increase in concentration at 3 sites. Higher concentrations of microplastics were observed in surface water sampled from the outflow sites on 4 of 7 rivers during the first campaign and 5 of 7 rivers during the second campaign (Fig. 3-4; Fig. 3-5). Microfibres represented the majority of microplastics found for every site for both years, with a range of; 51.4% - 90.6% and 67.4% - 93.6% for the 1st and 2nd sampling campaigns (Fig. 3-6). In general, there was a low abundance of microbeads recovered from both sampling campaigns. With the exception of the Fane Outflow location where 13.8% of microplastics recovered were microbeads, microbeads represented a maximum of 5.5% of the total microplastics recovered in the first sampling regime (range: 0% - 13.8%). For the 2nd sampling regime there was a range of 0% - 6.9% for microbeads recovered per site (Fig. 5).

Although microplastics were recovered for all surface water samples, a significant median microplastic concentration variation between the two sampling campaigns was observed (Mann-Whitney U, $W = 257$, $P = 0.014$). Samples collected from the first campaign (mean = 1.6 ± 2.24 MPs L^{-1}, median 0.81 MPs L^{-1}) had a significantly higher concentration than those collected in the second sampling campaign (mean = 1.14 ± 1.74 MPs L^{-1}, median = 0.45 MPs L^{-1}). No significant difference was found, however, when comparing median microplastic concentrations for combined years between headwater and outflow sites (Mann-Whitney U, $W = 178$, $P = 0.260$) or the first and second sampling campaigns individually (Mann-Whitney U, $W = 45$, $P = 0.371$, Mann-Whitney U, $W = 47$, $P = 0.522$) respectively. For the combined sampling campaigns headwater sites were found to have a lower mean (0.62 ± 0.25; range:

0.33 – 1.01 MPs L^{-1}) and median microplastic concentration (0.61 MPs L^{-1}) than the outflow sites assessed (mean: 2.02 ± 2.63; range: 0.23 – 8.55 MPs L^{-1}; median; 0.74 MPs L^{-1}). Kruskal-Wallis tests revealed that there were no significant differences in the concentration of microplastics of individual sites for either the first or second sampling campaign respectively ($P = 0.452$, $P = 0.45$).

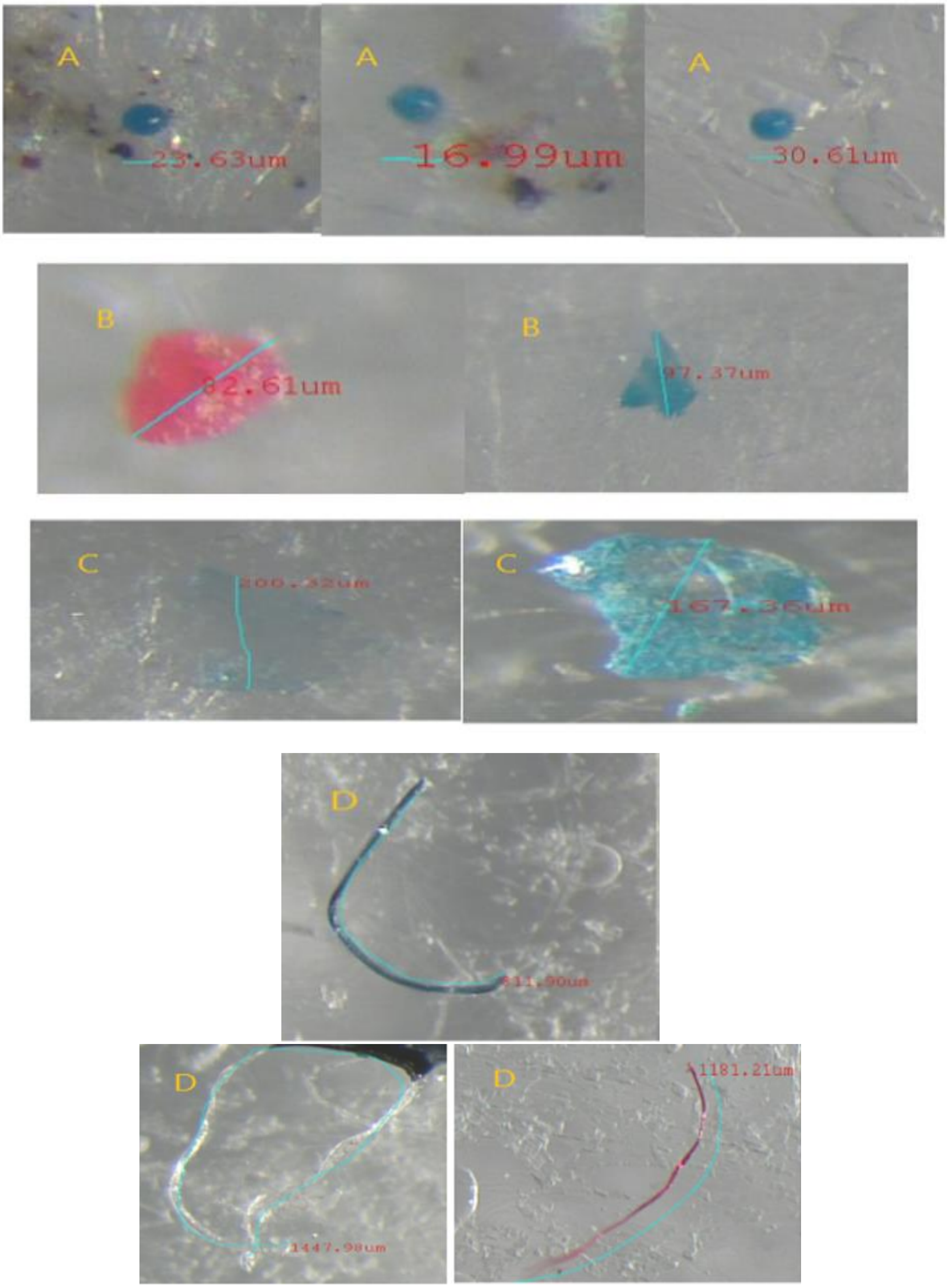


Figure 3-3: Microplastics recovered in surface water; A: microbeads, B: fragments, C: fibres.

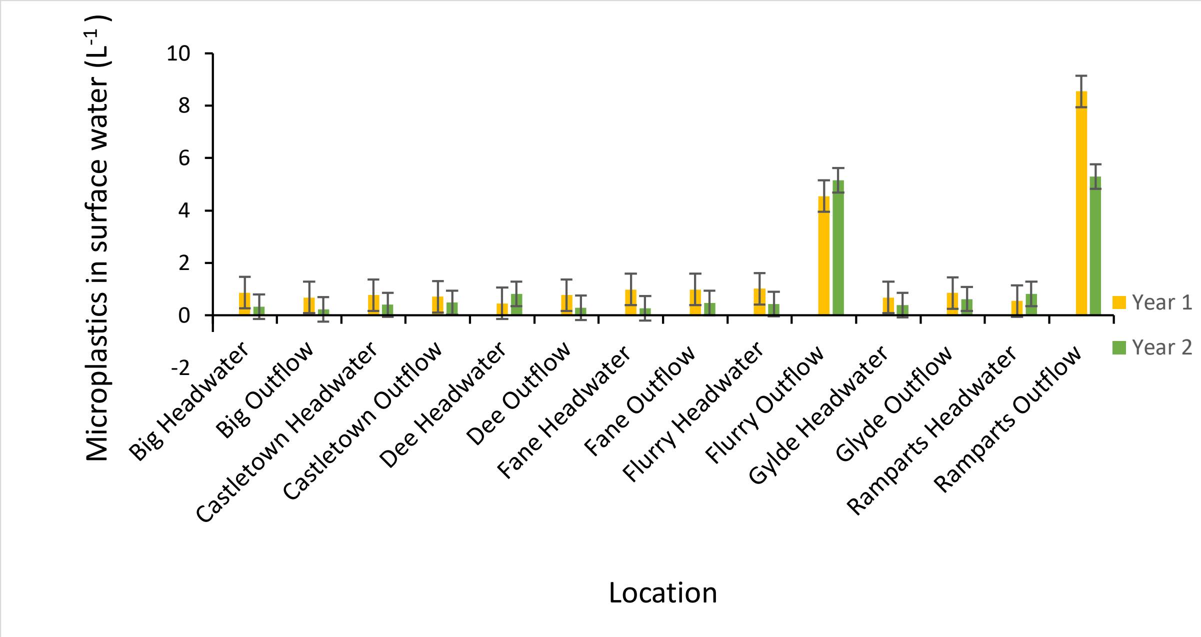


Figure 3-4: Comparative microplastic concentrations (MPs L^{-1}) in surface water for rivers flowing into Dundalk Bay for both sampling campaigns.

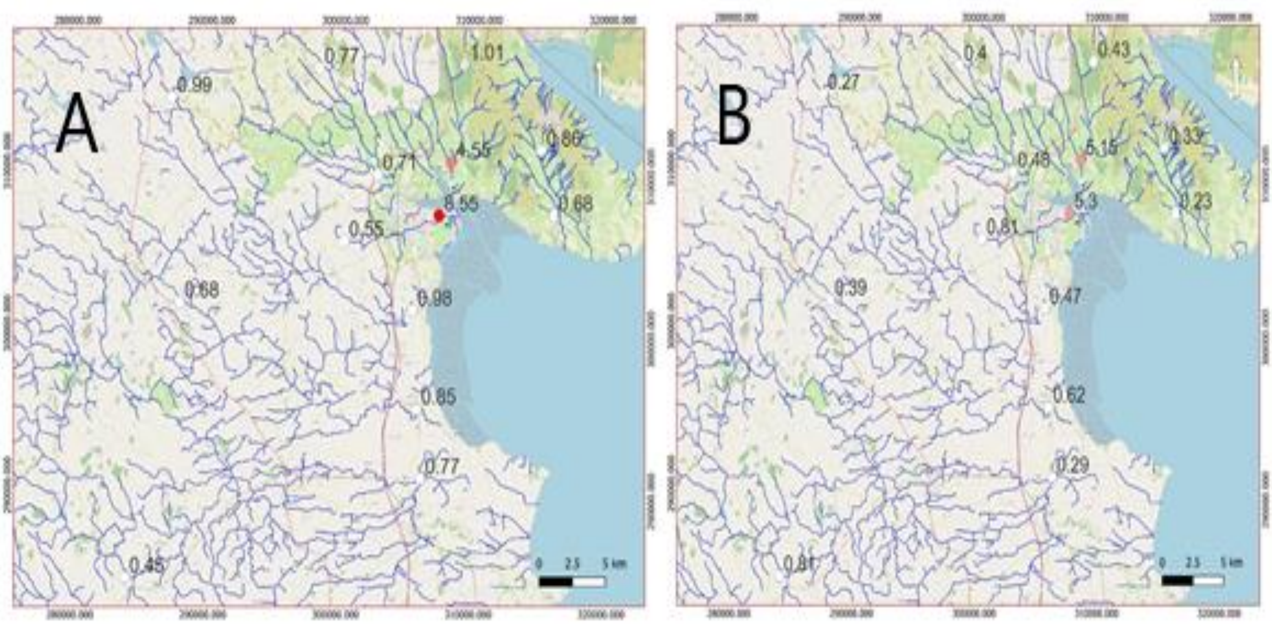
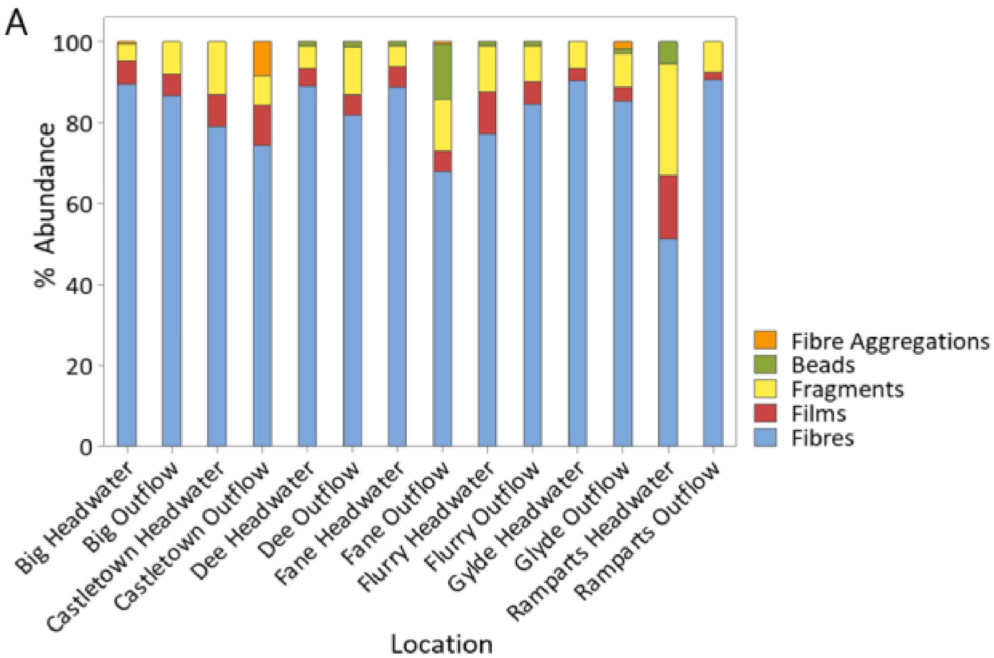


Figure 3-5: Microplastic concentrations (MPs L^{-1}) of surface waters in rivers that enter Dundalk Bay for first sampling campaign, A and second sampling campaign, B.

There was a similar colour profile of microplastics recovered from the surface water of rivers sampled in this study between campaigns. For the first sampling campaign the colour breakdown was as follows: blue (31.3%), transparent/white (30.5%), red (12.2%), black (11.7%) with the remaining 14.3% made up of green, multi-coloured and other colours. The colours of microplastics from the second sampling campaign were as follows:

transparent/white (25.3%), blue (24.2%), black (20.5%), red (16.7%) with green, multi-coloured and other colours making up the remaining 13.3%. Microplastics <1mm in size made up the majority of microplastics recovered for both sampling campaign one and two consisting of 64.4% and 62.7% of the total recovered respectively. Microplastics in the smaller category (<300µm) consisted of an important component in size distribution of those recovered from the 14 sites with a range of 12.8% - 45.9% and 14.1% - 37.0% for the 1st and 2nd sampling campaign respectively.



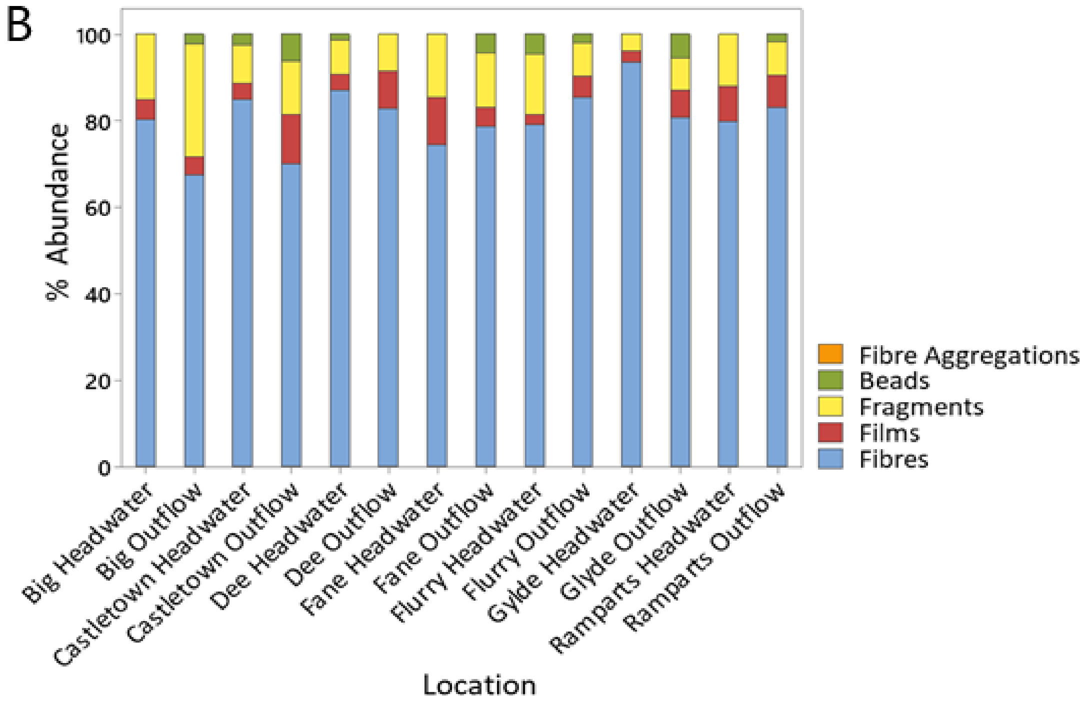


Figure 3-6: Shape profile of microplastics found in surface water for first sampling campaign; A and for second sampling campaign B.

3.4.2 Polymer composition of microplastics recovered from surface water

A subsample of suspected microplastics was analysed using Raman spectroscopy from surface water samples (n = 344, 10%) which resulted in positive matches for 277 suspected microplastics. The majority of those selected were identified as synthetic in nature (Fig. 6). Cellulose and mineral fragments constituted 9% of the total analysed. For 19% of those identified the underlying polymer was masked by the presence of dyes such as indigo and pigment violet 23 which are anthropogenic compounds. Interestingly some polymers such as polyamides and polyesters with densities greater than that of freshwater and would be expected to sink were noted. The proportion of polyesters identified between campaigns was similar, however, there was a greater proportion of dyes identified in the second campaign. Additionally, PP which constituted 8% of microplastics identified in the first campaign was not identified amongst material in the second campaign. While there was a similar spread of polymers found between headwater and outflow locations there was a greater proportion of PA and polyesters in outflow locations, while cellulose and dyes constituted a greater proportion at headwater sites.

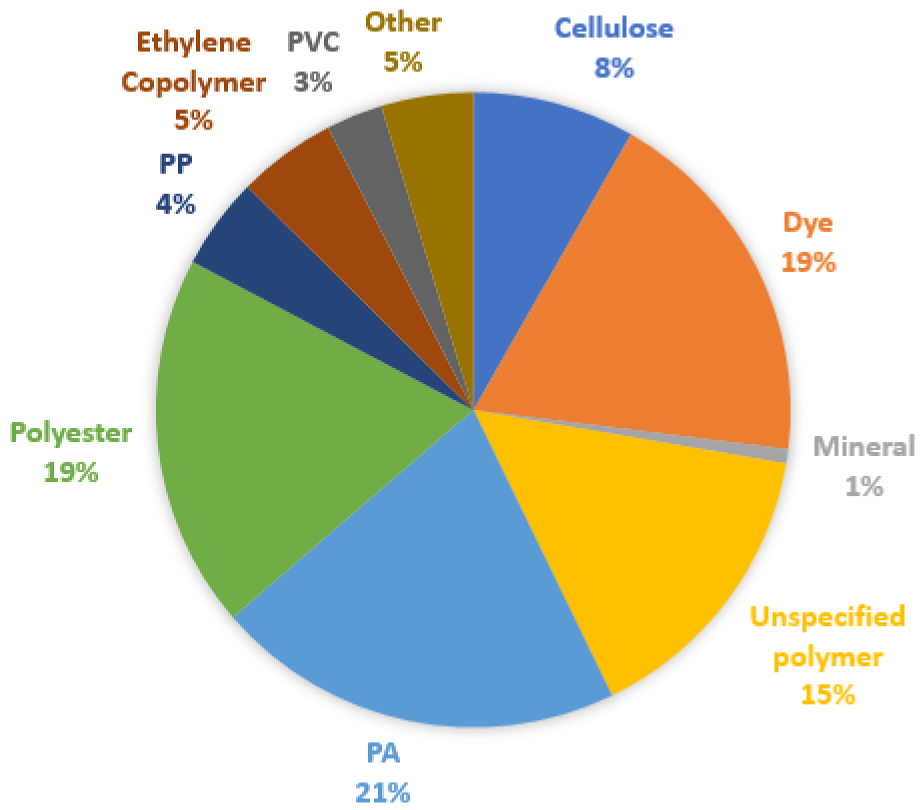


Figure 3-7: Composition of material recovered from surface water samples for both sampling campaigns identified using Raman Spectroscopy.

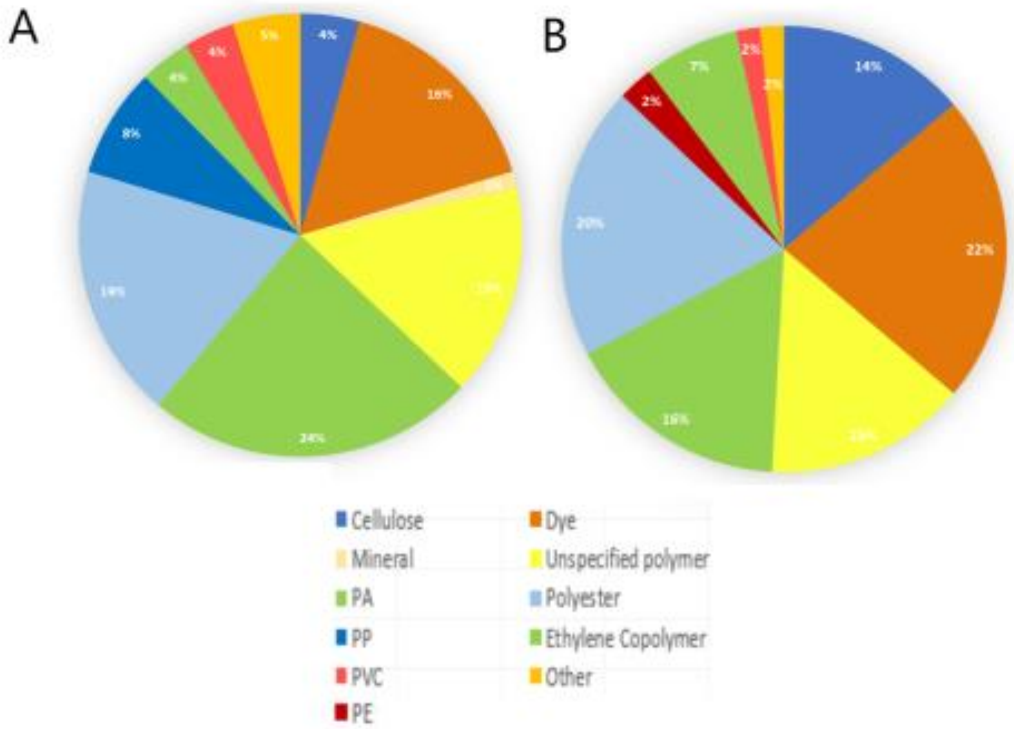


Figure 3-8: Comparison of identified material between both sampling campaigns. A; first sampling campaign. B; second sampling campaign.

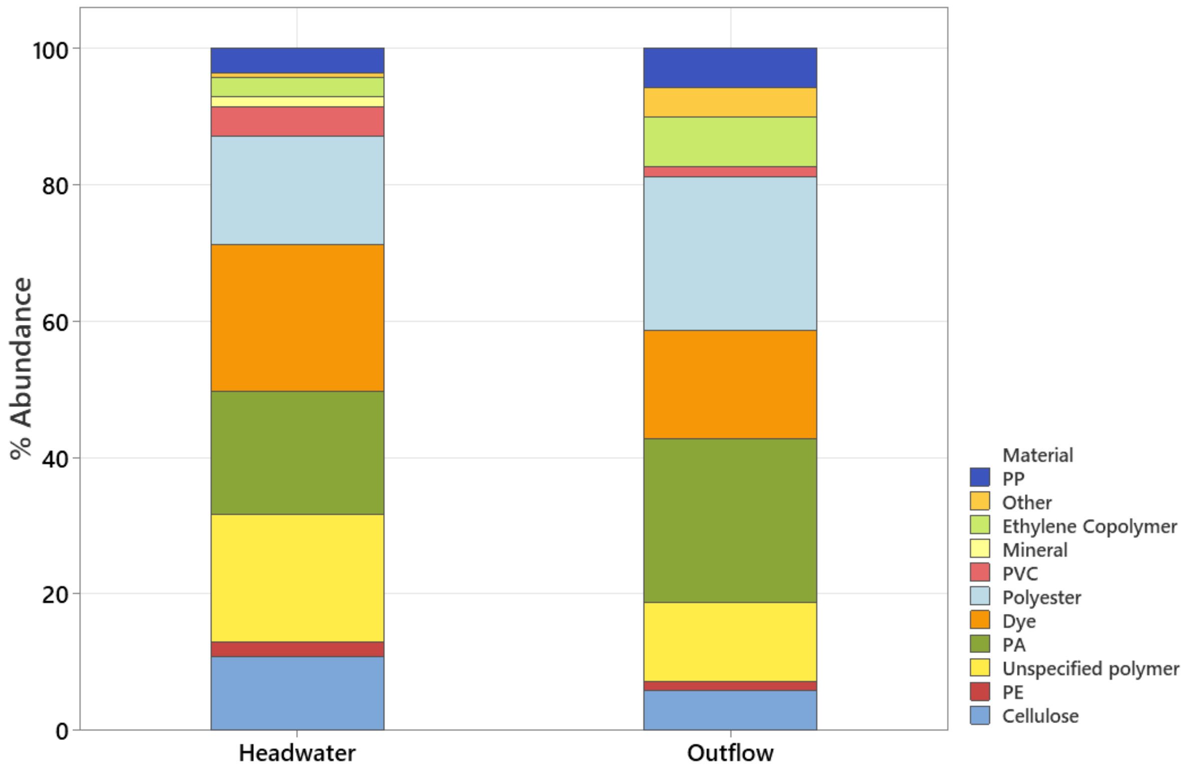


Figure 3-9: Comparison of abundances of polymers identified between headwater and outflow sites for both years.

3.4.3 Microplastics in sediment and *G. duebeni*

Microplastics were recovered from both headwater and outflow sediment samples taken on the River Flurry. Two sample t-tests revealed a significant difference between the mean concentrations of microplastics in sediment of the two sampling locations ($P = 0.007$). The downstream location had a higher median microplastic concentration (370 MPs kg^{-1}) than the upstream location (180 MPs kg^{-1}) as well as a greater mean microplastic concentration (downstream: 395 ± 75.5 MPs kg^{-1}; range: 340 – 500 MPs kg^{-1}, upstream: 190 ± 52.9 MPs kg^{-1}; range: 140 to 260 MPs kg^{-1}) respectively.

Two sample t-tests revealed there was no statistically significant differences found between composite samples of *G. duebeni* collected from the headwater or outflow sample sites with regards to microplastic concentration mg^{-1} ($P = 0.062$) or microplastics $composite^{-1}$ ($P = 0.067$). From *G. duebeni* examined from the outflow site 101 microplastics were noted (2.02 MPs $composite^{-1}$) while 41 were present in *G. duebeni* sampled from the headwater location (1.36 MPs $composite^{-1}$). However, there was a small difference in microplastic levels in *G. duebeni* specimens between the two sites, 0.059 MPs $mgtissue^{-1}$ (headwater) and 0.052 MPs $mgtissue^{-1}$ (outflow) respectively.

Microplastic characteristics were compared between the water samples from the second sampling campaign and those found in *G. duebeni* and riverbank sediment to evaluate how closely these other environmental matrices reflected those present in water. The shape profile of microplastics recovered in sediment from both locations was similar to that of *G. duebeni* sampled from both sites. Microfibres made up the majority of microplastics recovered in both sediment and *G. duebeni* from the downstream location consisting of 87% and 69% recovered in both matrices with the remainder consisting of fragments, 13% and 31% respectively. Similarly, microfibres were the dominant shape profile found in both sediment and *G. duebeni* at the headwater site (76% and 85% respectively), while fragments were found in both matrices (5% and 15% of total). Films however were only present in sediment samples from the headwater location and consisted of 19% of the total microplastics recovered. Microfibres also made up the majority of the microplastics recovered in surface water from the previous sampling campaign for both headwater (79%) and outflow / downstream sites (85%) closely following the trend of the sediment in particular, however shape abundances of microplastics varied between matrices for other shape profiles (Table. 3-3).

Table 3-3: Comparison of microplastic shape compositions and relative abundances for water, *G. duebeni* and sediment samples.

Shape	Flurry Headwater water	*G. duebeni* Headwater	Flurry headwater sediment	Flurry Outflow Water	*G. duebeni* Outflow	Flurry outflow sediment
Fibre	79%	85%	76%	85%	69%	87%
Film	2%	0%	19%	5%	0%	0%
Fragment	14%	15%	5%	8%	31%	13%
Bead	5%	0%	0%	2%	0%	0%

White / transparent microplastics were the most commonly recorded for both headwater and outflow sediment samples accounting for 76% and 75% of total microplastics respectively, however, they did not constitute a large proportion of those recovered from water samples in the previous campaign for either headwater (19%) or outflow locations (24%). In sediment, black microplastics accounted for 11% of the total microplastics recovered from both sampling locations while there was a greater spread of colours recovered from the downstream sediment than the upstream site with red, blue, green and other colours detected. In contrast to the colour breakdown found in riverbank sediment and water samples, blue microplastics were the most common recovered from *G. duebeni* for both upstream and downstream locations (60% and 38%) which was followed by black (25.7% and 32.6%) (Fig. 3-10). For all matrices examined white / transparent microplastics made up the smallest proportion of those found in *G. duebeni* tissue. There was a large difference in the colour composition of microplastics present in G. duebeni compared to that observed in water samples from the river Flurry. Blue and black microplastics made up a combined 86% of those observed in *G. duebeni* at the headwater location and 71% at the outflow location. Conversely, these made up just 44% of the microplastics in surface water at the headwater location and 46% at the outflow location.

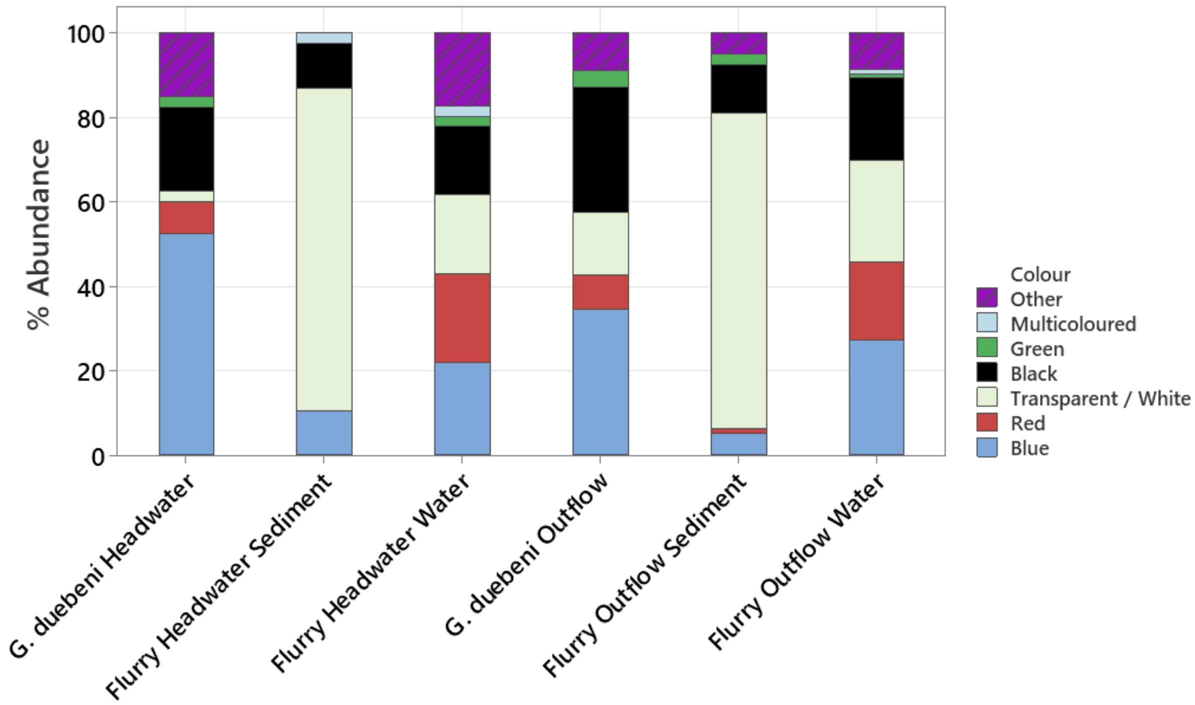


Figure 3-10: Colour profile of microplastics recovered in surface water from the second sampling campaign compared with those recovered in G. *duebeni* and sediment.

Microplastics in the <300µm in size consisted of higher percentages of the total found in *G. duebeni* from both sites than those recovered in sediment or water samples. A smaller proportion of microplastics less <300µm were recovered in sediment from either sampling site than were present in water samples taken in sampling campaign two. The size ranges of microplastics recovered from both sediment and *G. duebeni* samples are displayed below (Fig. 3-11).

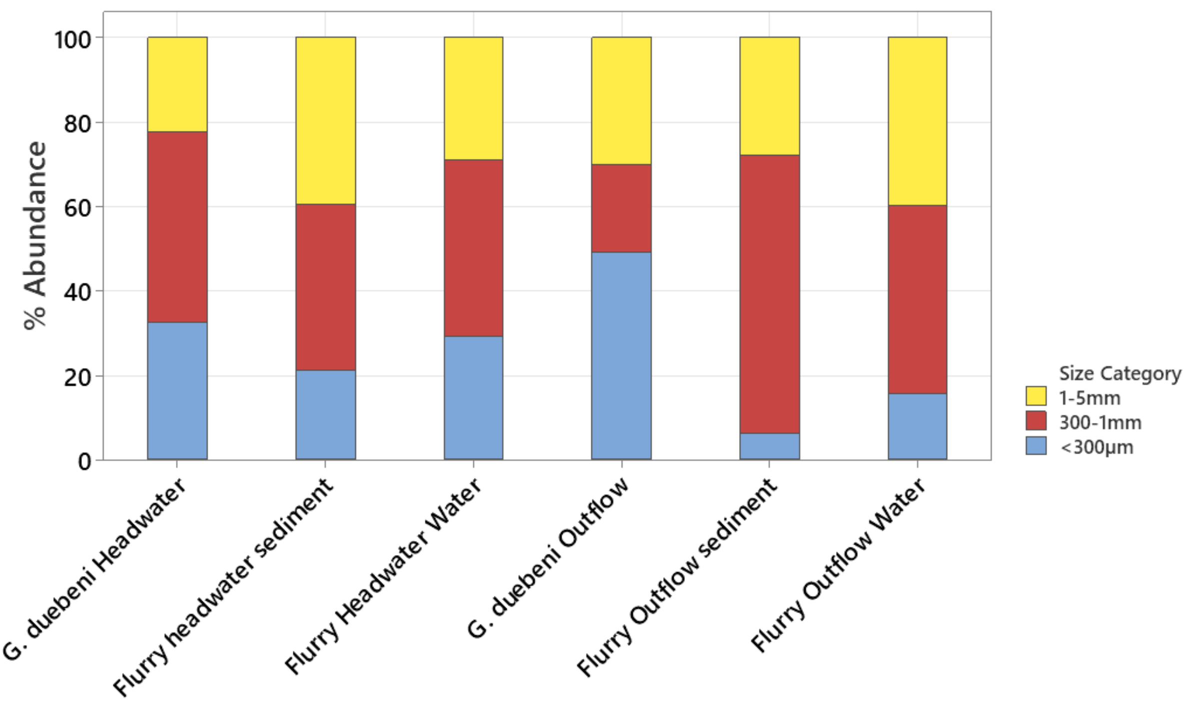


Figure 3-11: Comparison of microplastic size profiles between the three environmental compartments studied on the river Flurry.

Raman analysis was conducted on a subsample of suspected microplastics from sediment (n = 50, 43%) with positive matches identified for 37 items. There was a greater spread of polymers identified in sediment collected from the outflow site than the headwater site on the River Flurry with 9 polymers positively identified. For both sites polyamide was the dominant polymer identified and cellulose was only present sediment collected from the outflow location (Fig. 3-12). A subsample of suspected microplastics (n = 45, 31%) were selected for Raman analysis from *G. duebeni* of which positive matches were determined for 35 items. As noted in sediment samples there was also a greater spread of anthropogenic material identified in *G. duebeni* samples collected from the outflow location in comparison to the headwater location. Dyes made up a greater proportion of positively identified material (likely masking underlying polymers identity) in *G. duebeni* found at both locations than that identified in river water or sediment, highlighting a possible preference for this type of material. An unspecified polymer described in chapter 2 (section 2.9.2) of this thesis was also detected with greater frequency in *G. duebeni* than in water samples and sediment and is likely polyamide and some additive (Fig. 3-13). Examples of polymers identified from the three difference environmental matrices are shown (Fig. 3-14)

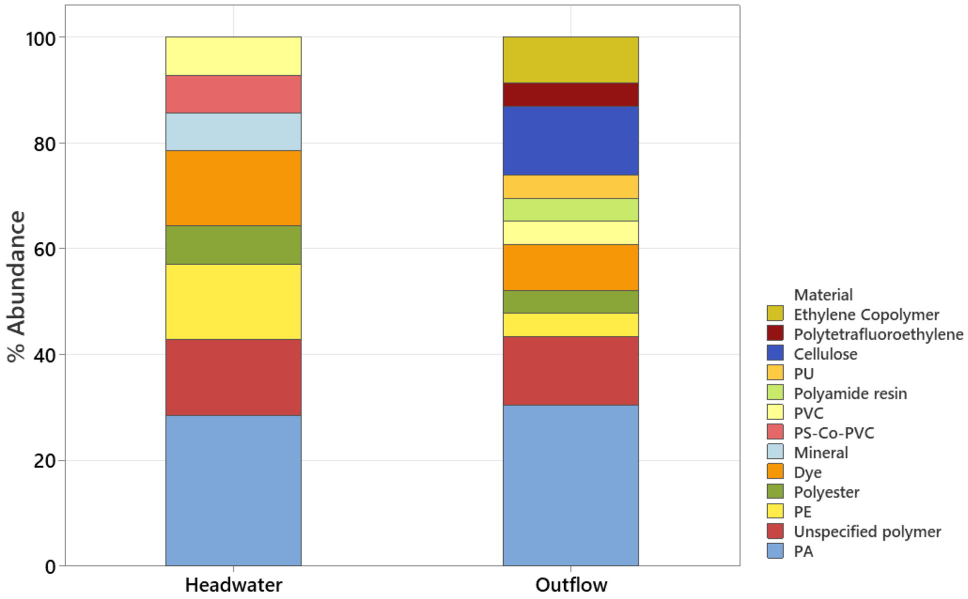


Figure 3-12: Polymer composition in riverbank sediment sampled from both locations on the river Flurry.

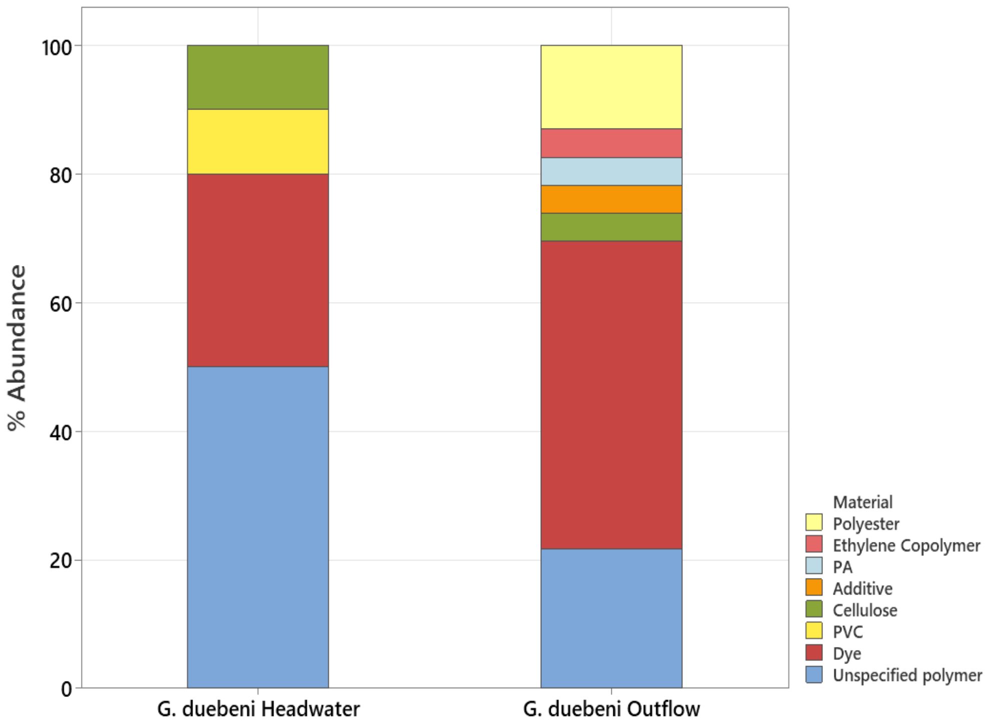


Figure 3-13: Polymer composition of material recovered from *G. duebeni* from both locations on the river Flurry.

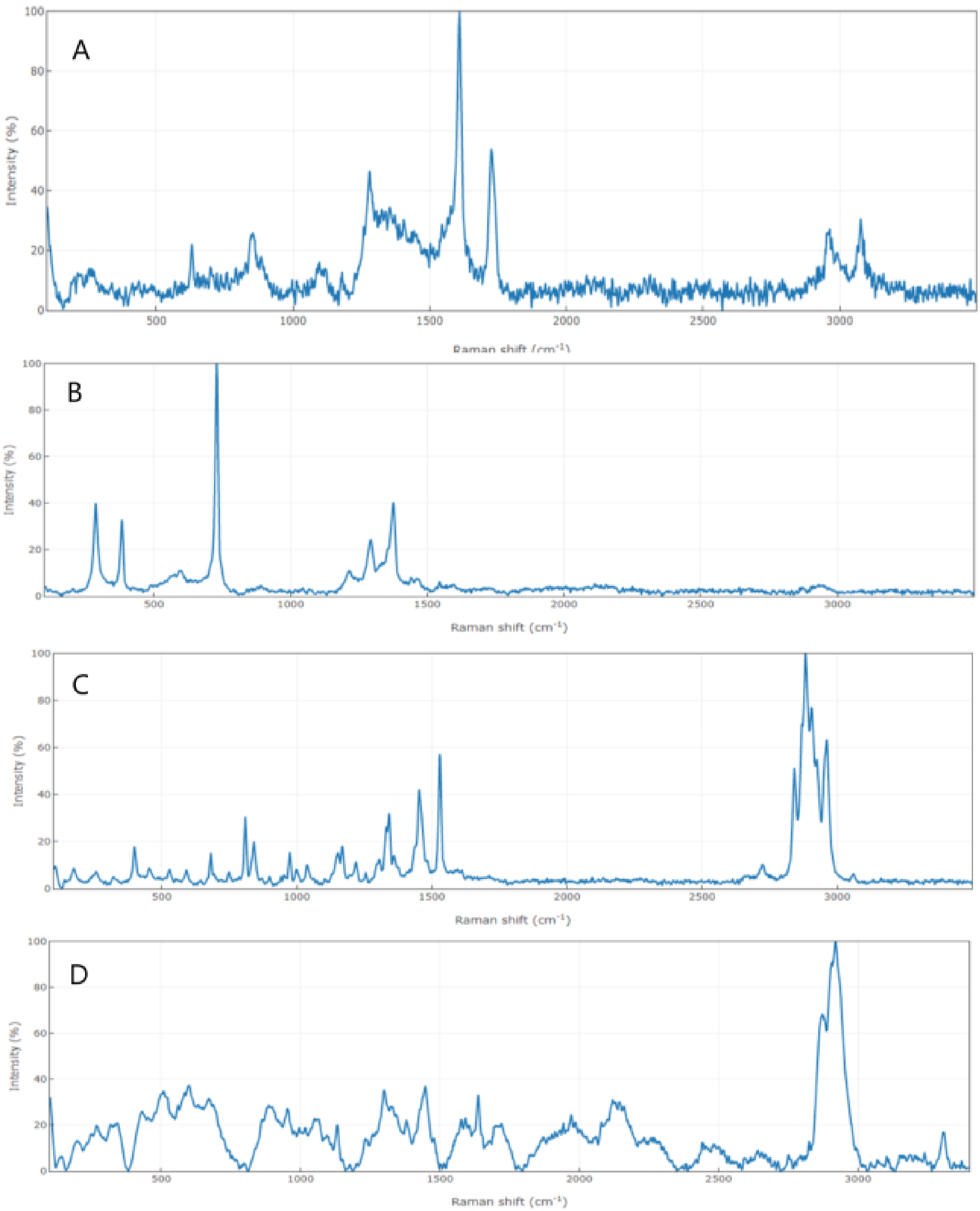


Figure 3-14: Raman spectrum of identified polymers. A: polyester from *G. duebeni*, B: polytetrafluoroethylene from sediment, C: polypropylene from surface water, D: polyamide from sediment.

3.5 Discussion

The concentrations of microplastics found in this study (sampling campaign one: 0.45 – 8.55 MPs L^{-1}, sampling campaign two: 0.23 – 5.30 MPs L^{-1}) are compared to other European studies in the table that follows (Table. 3-4).

Table 3-4: Microplastics levels present in rivers studied compared to other European rivers.

Rivers	Location	MPs L^{-1}	Reference
The Castletown, Flurry, ramparts, Dee, Glyde, Fane, Big	North-East Coast of Ireland	0.45 – 8.55, 0.23 – 5.30	Current Study
The Ebro	Spain	0.0035 ± 0.0014 $^{-1}$	Simon-Sánchez *et al*. (2019)
The Elbe	Germany	0.00557	Scherer *et al*. (2020)
The Trent, Leen, Soar	United Kingdom	0.019 – 0.083	Stanton *et al*. (2020)
The Ergene	Turkey	4.65 ± 2.06, 6.90 ± 5.16	Akdogan *et al*. (2023)
The Thames	United Kingdom	10.91 – 18.83	Devereux *et al*. (2023)
The Meuse and Dommel	The Netherlands	0.067 – 11.532	Mintenig *et al*. (2020)
The Antua	Portugal	0.058 – 1.265	Rodrigues *et al*. (2018)

As noted by Matjašič *et al*. (2023) most freshwater studies on microplastics have investigated pollution patterns in larger rivers (catchment area > 50,000 km^2 or main tributaries with catchment areas > 5000 km^2). Furthermore, it has been observed that small and medium-sized rather than larger rivers can have high microplastic levels (Schere *et al*. 2020; Heß *et al*. 2018). The comparatively high levels of microplastics found in some of the rivers examined in this study; namely the Flurry and Ramparts underline the importance of considering small streams and rivers as contributors of microplastic pollution to marine environments.

The predominance of microfibres for every sample taken across all matrices assessed is not surprising and is similar to the results of other studies. A review of freshwater studies on microplastic presence found that microfibres constituted 59% of total microplastic shapes recovered which was followed by fragments at 20% (Li *et al*. 2020). In the Rivers Rhine and the Danube fibres and fragments were the dominant shape of microplastics found in the water phase (Heß *et al*. 2018), however, microplastic spheres and pellets dominated in other studies, possibly due to nearby plastic production (Mani *et al*. 2015; Lechner *et al*. 2014). The dominance of microfibres in general in freshwaters is potentially due to the release of greywater from domestic settings or their escaping septic tanks (Le *et al*. 2022; Liu *et al*. 2022). Microfibres end up in greywater following the washing of textiles in domestic settings due to the mechanical and chemical stresses that clothes undergo in washing machines that have been shown to release millions of particles with increased washing load weight (Volgare *et al*. 2021). Additionally, microfibres can be dispersed due to precipitation especially in urban areas (Dris *et al*. 2016).

A significant difference in microplastic concentrations was observed between first and second sampling campaigns which may be attributed to the increased rainfall during the first sampling campaign. The total rainfall recorded at the nearest weather station in the study location for the first sampling campaign was 157.8mm while for the second it was approximately 122.8mm (Met Éireann, 2020, 2021). Ideally, this would be better examined as a factor for microplastic abundances between a dry season and wet season, however, time constraints and the onset of Covid-19 made this impossible to conduct. Previous studies have linked wet periods and precipitation to increased microplastic presence in freshwater bodies when compared to drier spells (Xia *et al*. 2020; Piñon-Colin *et al*. 2020; Wong *et al*. 2020). Increased microplastics in inland waters in China after periods of rainfall were stated as probably due to increased runoff from land which can deliver microplastics into waterbodies (Xia *et al*. 2020). Furthermore, rainfall can directly affect the microplastic abundance in the surface water of rivers, as previous work reported that there were plenty of microplastics floating in the atmosphere (Klein and Fischer, 2019; Gasperi *et al*. 2018), microplastics can adhere to raindrops and land in river bodies directly. Temporal variation has been documented where higher microplastic concentrations have been observed in surface waters following rainfall in the past (Campanale *et al*, 2020).

Microplastics in surface waters have also been correlated with urbanisation and population density in previous studies on river systems (Kataoka *et al*. 2019; Yonkos *et al*. 2014; Mai *et al*. 2021). A similar trend could be seen in this current study, microplastic levels were highest for both sampling campaigns at the outflow site on the Ramparts Rivers (5.30 and 8.55 MPs L^{-1}). This site is located just past the urban centre of Dundalk which has the highest population density of locations studied as part of this work. The headwater site of the Ramparts River which is located in a rural area (Kilkerley) had much lower microplastic levels in its surface water and was consistent between sampling campaigns (0.55 and 0.81 MPs L^{-1}). These two sites are located in exact opposites in the Irish settlement hierarchy classing (road network, population density etc.) for County Louth; Dundalk classed as a 'Regional Growth Centre' and Kilkerley classed as a 'Rural Node'. This increased microplastic presence in urban areas has been linked to littering and insufficient waste management strategies in the past (Battulga *et al*. 2019; Mani and Burkhardt-Holm, 2020). A study on the Rhine River in Germany found that microplastic concentration increases with its flow towards the sea with the exception of the tidal zone (Mani *et al*. 2015) and this proved to be the case for 9 of the total 14 study sites from the two sampling campaigns. Although no significant differences were detected for either sampling campaign at individual sites, there was a biological difference noted with the concentrations of microplastics recorded at the Ramparts and Flurry headwaters higher than other sites studied.

For both marine and freshwater environments, the proximity to sources of microplastics (effluent pipes, urban runoff and septic systems) can impact on the amount of plastic found at a sample location (Carr *et al*. 2016). It is suspected that microplastics in the aquatic environment originate from WWTPs and large-scale urban development along freshwater rivers (Eerkes-Medrano *et al*. 2015, Conley *et al*. 2019). There is a likelihood that greater numbers of microplastics may be found at freshwater sampling locations near urban areas than at rural sites due to their proximity to sources of microplastics and this proved to be the case for the Ramparts outflow location in this study. However, the comparatively high microplastic abundances located at the outflow site on the River Flurry cannot be attributed to population density pressures or urban land use. This site although located in a rural area had a comparable level of microplastics found in its surface waters as the outflow site of the Ramparts River for both sampling campaigns (4.55 and 5.15 MPs L^{-1}). The sampling point is located between land classed as agricultural areas and next to a road on one side and artificial non-agricultural vegetated areas and sports and leisure facilities on the other side. There is an unclear link between microplastic pollution of water bodies and agriculture (Talbot and Chang, 2022), and while road surface proximity to water bodies is a known source of microplastics (Kallenbach *et al*. 2021) the high level found for both sampling dates may be due to the low elevation of the site (approximately 15 metres above sea level) when compared to its headwater site (approximately 330 metres above sea level) (Fig. 3-15). Other sites located on the same river courses studied as part of this work were located on agricultural land and close to roads and did not display this high concentration for surface water microplastics while having a maximum elevation difference between sampling locations of less than 50 metres. The large difference in elevation between sites on this river may account for the increased surface microplastic levels at the outflow site due to increased washing and surface run-off of microplastics as it flows. Notably, physical catchment characteristics and river morphology can influence microplastic presence (elevation, slope etc.) however few studies have directly addressed these links (Talbot and Chang, 2022). Increased slope of the riparian zone has been correlated with increased microplastic presence in surface water samples previously (Grbić *et al*. 2020), while in Australian water bodies higher microplastic concentrations were found in those located at lower elevations (Su *et al*. 2020).

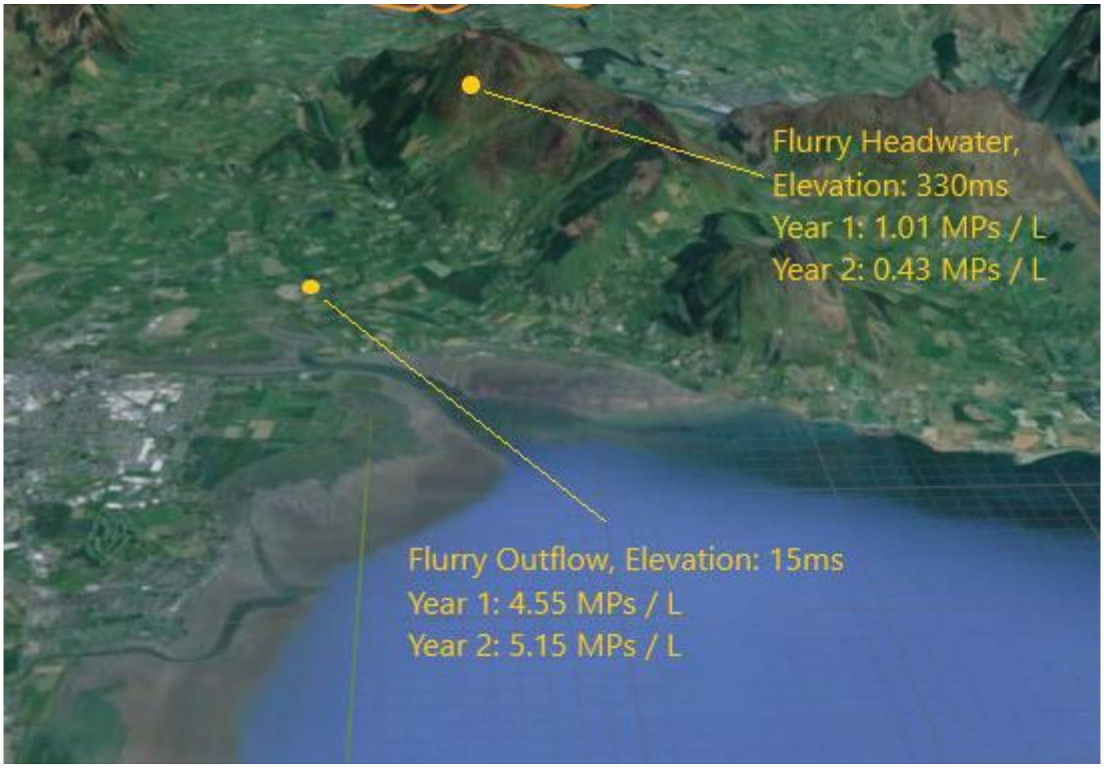


Figure 3-15: Comparison of microplastic concentrations in surface waters at headwater and outflow sites on river Flurry and associated elevations.

The use of stacked sieves with mesh sizes down to 50µm enabled the capture of microplastics of smaller sizes in this study. The majority of microplastic freshwater studies on rivers have used trawls or nets to collect water samples, generally with a mesh size of 330µm or larger (Deocaris *et al*. 2019; Campanale *et al*. 2020; Katoaka *et al*. 2019). Smaller microplastics (<300µm) accounted for a range of 12.8% - 45.9% and 14.1% - 37.0% of total microplastics captured for the 1st and 2nd sampling campaigns per river in the current study respectively, thus highlighting the importance of using equipment with smaller mesh sizes. Unless the sieves or nets clog, microplastics under the 330µm will be missed. This lower size range being particularly important as a size bioavailable to zooplankton (Botterell *et al*. 2019). As microplastics decrease in size so too can their bioavailability (Vroom *et al*. 2017) and it is therefore of paramount importance to quantify the levels present of smaller microplastics in Irish freshwater environments. Similarly, to the findings of Walkingshaw *et al*. (2022) larger microplastics in particular microfibres were found on smaller mesh sieves and it has been noted that this can be due to inconsistencies in mesh size across the filter / sieve or due to fibres small width they can pass through coarser sieves when orientated correctly (Barrows *et al*. 2017; Coverton *et al*. 2019). Microplastics smaller than the smallest mesh size used (<50µm), notably microbeads and fragments were recovered in this study which was not expected. Their presence may be due to them becoming trapped in aggregations of organic material, tangling with microfibres, becoming trapped later in the sampling process as meshes became blocked or adhered to other material caught on the sieves.

Microplastics presence in sediment was lower at the headwater site of the river Flurry (195 MPsKg^{-1}) than at the outflow site (395 MPsKg^{-1}). In an Irish context the concentrations of microplastics recovered from sediment in this study exceed the upper limit on the range reported for sediment from the river Barrow (155 MPsKg^{-1}) (Murphy *et al*. 2022). Similar to the current study fibres were the dominant shape found and red, blue, white and black the dominant colours found in River Barrow sediment (Murphy *et al*. 2022). Particles less than 1mm in size were the dominant size category recovered from both headwater and outflow locations in this current study (60.5% and 72.2% respectively) and is in line with the size categories normally recovered from freshwater sediment (Yang *et al*. 2021). The suite of polymers recovered in sediment in this study had 2 polymers (PET and nylon (PA)) in common with another study that examined sediment in Irish rivers (Murphy *et al*. 2022).

A wide range of microplastic concentrations is reported for freshwater sediment in European rivers. Very low contamination of sediment was reported in the Carpathian basin, Hungary with a range of 0.46 to 1.62 particlesKg^{-1} (Bordo *et al*. 2021). Similarly, the lowest concentration of microplastics in sediment from the Antuá River, Portugal was 18 items kg^{-1}, however, the upper limit reported was much higher at 629 items kg^{-1} (Rodrigues *et al*. 2018). The concentration of microplastics reported at the outflow site of the River Flurry surpass the lower limit of some other studies on riverine sediment pollution, namely; the Rhine-Main River, 228 – 3763 items kg^{-1} and the Rhine River 260 ± 10 to 11,070 ± 600 items kg^{-1} (Klein *et al*. 2015; Mani *et al*. 2019). Other studies in Europe have reported higher concentrations in riverine sediment namely; The Thames River, UK (66 items g^{-1}) (Horton *et al*. 2017) and an urban recipient in Norway (12,000 – 200,000 items kg^{-1}) (Haave *et al*. 2019).

The use of potassium carbonate (K_2CO_3) for density extraction from riverbank sediment was advantageous as it is non-toxic and cheap (Gohla *et al*. 2021) which increases its usability for citizen science projects and therefore increases the viability of long-term monitoring of sediment in freshwater bodies. Furthermore, a wide range of polymers were identified from sediment samples with densities ranging 0.9-1.0 g/cm^3 for polyethylene to 1.3-1.4 g/cm^3 for polyester (Li *et al*. 2018) and 1.5 g/cm^3 for cellulose thus highlighting the efficacy of extraction for environmental samples.

The presence of microplastics particularly of microfibre shape profile in *G. duebeni* found in this study is evidence of the consumption of these microfibres in the natural environment. *G. duebeni* have been shown to consume microfibres in a lab-based study in both the presence and absence of food (Mateos-Cárdenas *et al*. 2021). This was displayed, however, in much higher concentrations than those found in the previous two sampling campaigns on the river Flurry (600 microfibres mL^{-1}) (Mateos-Cárdenas *et al*. 2021).

The higher abundance of microplastics <300µm in *G. duebeni* than found in surface water samples or riverbank sediment may indicate that these are being broken down to smaller sizes when ingested. While *G. duebeni* have been shown to fragment microplastics to smaller sizes in the past, no evidence of fragmentation of polyester or cellulose microfibres have been documented (Mateos-Cárdenas *et al*. 2020; Mateos-Cárdenas *et al*. 2021). Further evidence of the shredding nature of this species for microplastics was the lack of films found in their tissue which may be due to feeding of *G. duebeni* increasing the coarseness of ingested particles, with films having a smoother more defined thin and broad surface than irregularly shaped fragments. It must also be remembered that the smallest sieve used to extract microplastics from water samples was 50μm in size and may have accounted for this observed difference, furthermore, when attempting to quantify small particles < 50μm in length there can be error introduced (Simmerman and Wasik, 2019).

Microplastics have been documented in several other species of freshwater macroinvertebrates. *Baetidae*, *Heptageniidae* and *Hydropsychidae* had concentrations ranging up to 0.14 MP mg tissue^{-1} much lower than those reported in this study despite the fact they were taken from sample sites near highly urbanised areas (Windsor *et al*. 2019). In contrast, microplastics were reported in much higher concentrations (129 ± 65.4 particles g^{-1} tissue) in freshwater Tubifex worms in a major urban waterbody fed by the River Irwell, Manchester, UK (Hurley *et al*. 2017). The mayflies (*Heptageniidae*), caddisflies (*Hydropsychidae*), and amphipods (*Gammaridae*) had a mean MPs g^{-1} tissue of 15.1 ± 10.0 in sites at a city limit but this decreased to a mean MPs g^{-1} tissue of 5.5 ± 3.1 in upstream locations (Simmerman and Wasik 2019). Hurley *et al*. (2017) noted that microfibres represented the most abundant shape of microplastic found in Tubifex worms constituting 87% of the total with fragments making up the remaining 13% which was similar to the shape profile ingested by *G. duebeni* in this study (headwater: 85% microfibres, 15% fragments, outflow: 69% microfibres, 31% fragments). Microfibres were also the most commonly documented shape found in the bivalve, *Anodonta anatina* (Berglund *et al*. 2019) and dominated microplastic composition in the gastropod species: *L. varicus* (65.8%), *M. tuberculata* (100%), and *T. fluviatilis* (100%) with microplastic content ranging from 1.71 ± 0.46 g^{-1} to 6.1 ± 1.05 g^{-1} (Akindele *et al*. 2019). While both sites on the River Flurry were located in rural areas without population pressures contributing to elevated microplastic levels, high concentrations of microplastics were observed in the surface water of the outflow site on the River Flurry yet not noticed in sampled *G. duebeni*.. This may indicate that G. duebeni are not grazing on microplastics as frequently as other macroinvertebrate species are.

Interestingly, *G. duebeni* sampled from the outflow site had a lower proportion of microfibres in their tissue (69%) than was recovered in the surface water during the previous sampling campaigns (84.6%, 85.4%). The opposite was seen at the headwater site with microfibres consisting of 85% of microplastics recovered in *G. duebeni* but lower proportions found in surface waters (77.2%, 79%). However, this may be due to limitations in the dataset and long-term monitoring of both *G. duebeni* and water may be necessary to ascertain the true power of using this species as a reliable biomonitor for microplastics. *G. duebeni* were on average larger in size at the outflow site (38.5mg ± 320mg) than those at the headwater site (22.7mg ± 25.1mg). The higher proportion of microfibres found in smaller *G. duebeni* than larger ones in this study may be due to the mechanical limitations of the digestive tract accumulating microfibres for longer periods in smaller specimens (decreased gut size trapping microfibres). Microfibres have been shown to accumulate significantly more in the midgut-hindgut section compared to the foregut in short-term feeding experiments on this species in the past (Mateos-Cárdenas *et al*. 2021). While Mateos-Cárdenas *et al*. (2021) showed that microfibres accumulate in the gut of *G. duebeni*, the brine shrimp (*Artemia* sp.) exhibited limited ingestion of microfibres in the absence of food and fast egestion and no ingestion when in the presence of food (Bour *et al*. 2020). Furthermore, a study on the amphipod *Hyalella azteca* found that plastic microfibres were significantly more toxic than plastic microbeads which was attributed to a slower egestion rate of microfibres than beads (Au *et al*. 2015).

The larger abundances of microplastics coloured black or blue in combination with the frequent detection of dyes in *G. duebeni* in comparison to the proportions found in sediment and water samples indicate a preferential selection for these types of material. This may mean that colour may be an important cue for microplastic consumption in the natural environment for this species. The increased presence of blue and black microplastics in *G. duebeni* tissue may be due to predation of this more visible colour which may be similar to traditional prey items colours (green, brown, etc.) than transparent or white material. This could also be due to the presence of chemical dyes and their associated scents, as it is theorised that food detection and consumption is explained by chemosensory sensilla of scavenging amphipods (Havermans and Smetacek, 2018), however, a thorough study on preferential feeding based on colours is needed to verify this. This species has been shown to equally prey on food items contaminated with microplastics as ‘clean’ food items (Mateos-Cárdenas *et al*. 2022).

The presence of PA in all matrices examined indicates textiles are a significant source of microplastics to the freshwater environment associated with Dundalk Bay and likely stem from domestic washing sources as they are used in clothing, rugs and rope with other sources (Zhu *et al*. 2019). Polyamide has been shown to reduce the fitness in the first generation of freshwater non-biting midge *Chironomus riparius* and genetic adaptation to low microplastic concentrations (Khosrovyan *et al*. 2022), however despite their high abundance in other matrices examined they were identified only rarely in *G. duebeni* in this study.

The differences between both colour composition and in polymer types found in *G. duebeni* samples and water samples likely indicate that alone this species is not ideal as an indicator for microplastics pollution and will present a skewed subset of microplastics from the freshwater environment. G. duebeni displayed an apparent preferential ingestion of colured (blue / black microplastics) and those containing dyes rather than polyamide or polyesters which were the most commonly reported polymers found in water samples. For freshwater rivers the assessment of microplastics in a variety of macroinvertebrates is likely necessary to ascertain a true reflection of pollution in the surrounding environment.

The results of the microplastic composition found in riverbank sediment showing similarity to those noted in the previous water sampling campaign, in particular they were strongly related in terms of microfibre composition for both sites. In freshwater sediments fragments and fibers are typically the dominant shapes of microplastics, however, films can also dominate (Yuan *et al*. 2023). The high proportion of fibers found in the top layers of riverbank sediment compared to other shapes has been noted previously, however, there is a lack of studies examining the vertical distribution of microplastics with various shapes (Yuan *et al*. 2023). Piperagkas *et al*. (2019) documented the dominance of fibrous microplastics in the surface layer of Greek beaches in Northern Crete and fragments in deeper layers, implying that rounder and denser microplastics could enter sediment more easily than longer and lighter ones. Vertical coring would therefore be necessary to examine the distribution of microplastics to deeper layers in riverine sediment and verify this hypothesis for the freshwater environment. The much greater levels of microplastics found in riverbank sediment than surface water show the accumulation of microplastics at this terrestrial interface. The highest concentrations of microplastics are found along the shoreline and benthic sediments in freshwater environments (D'Avignon *et al*. 2021).

The presence of microplastics in the sediment, water and *G. duebeni* of the relatively remote and uphill site at the headwater location on the River Flurry may be due to the presence of nearby litter (Kurniawan *et al*. 2023) with the site a known hotspot for illegal dumping and is next to a road used for the movement of logging equipment and large vehicles (Vercauteren *et al*. 2023). Forestry which occurs nearby has also been linked to microplastics presence in the environment previously (Liu *et al*. 2022). Additionally, atmospheric microplastics transport and deposition driven by wind could account for some of the microplastics present at this more remote location with the long-range atmospheric transport of microplastics noted in urban and pristine locations in Southeast Asia having a deposition rate varying from 114 to 689 $MP/m^2/day$ (Hee *et al*. 2023).

Amongst polymers identified polyester and PA were the dominant found for both sampling campaigns in water samples and identified more commonly than lower-density plastics such as PP or PE. This occurrence in surface water may be attributed to hydrodynamical conditions causing resuspension of denser microplastics via turbulence in freshwater bodies and has been noticed before. The same was noted by Akdogan *et al*. (2023) in the Ergene River in Turkey where Polyethylene terephthalate (a polyester) and PA made up 55% of identified microplastics. Interestingly, the inverse was noted in pond sediments examined in Denmark, buoyant microplastics made up 83.5% of those recovered (Molazadeh *et al*. 2023). The high abundance of microfibres in the form of polyesters, including polyethylene terephthalate and PA in water samples found in this study likely stem from the washing of these materials in the catchment and entering the freshwater environment via greywater discharge or septic tank release as these are commonly used in textiles. Interestingly, lighter polymers namely, PP and PE were noted in the riverbank sediment at both the headwater and outflow study site the River Flurry, indicating that they may have been deposited here during flood conditions which may have occurred during the storm conditions present in February 2022 prior to sampling. Additionally, these microplastics have deposited here due to the washing of microplastics from land. The presence of microplastics in riverine sediment indicate that it serves as a sink for these contaminants, however, the exact length of time it does needs further study.

The prevalence of microplastics in these small order streams and rivers and observed in *G. duebeni* may be due to the habits of the Irish public in combination with current domestic wastewater infrastructure. Recent surveying commissioned by Uisce Éireann (Irish water utility company) found that one in five Irish adults are "using the toilet as a bin" – flushing wipes and other sanitary products which are sources of microplastics into the sewage network (Omorodion, 2023). A recent study into wipes labelled as "flushable" noted that 50% of tested brands contained a mixture of cellulose and microplastics in the form of PET (Ó'Briain *et al*. 2020). In combination with this is the fact that in 2021 over half of domestic septic tanks inspected failed countrywide. A 20% fail rate of domestic septic tanks was recorded in Louth with faulty septic tank systems capable of polluting rivers and posing issues for drinking water supplies (EPA, 2022). In Ireland there were 438,3019 individual septic tanks in use in 2016 (CSO, Domestic Waste Water Treatment Systems 2022). While recently a study in Flanders found an average daily release of 1145 microplastics through domestic wastewater equalling a yearly per capita discharge of 418,000 in the study area thus highlighting the impact this can have on the freshwater environment (Vercauteren *et al*. 2023). These factors together could explain the presence of microplastics in the rivers examined as part of this work. Further to this, the majority of Irish households and business are currently improperly disposing of their waste with two thirds of the waste going in the general waste bin that should be placed in recycling or composting units (EPA, 2023). This improper disposal of plastic instead of recycling leads it to be incinerated or sent to landfill with landfilling a known source of microplastics to the environment (Silva *et al*. 2021) and it was estimated that 11% of plastic waste generated globally in 2016 entered aquatic environments (Borrelle *et al*. 2020). Finally, the application of biosolids to agricultural lands adjacent to rivers may contribute to microplastics in freshwater bodies noted in this area. A previous Irish study examining microplastic presence in water near to biosolid application sites found 1.6 MPs L^{-1} in water from the sub-surface drainage outflow pipe and 0.3 MPs L^{-1} in groundwater (Heerey *et al*. 2023).

3.6 Conclusion

Currently the majority of microplastics research has been carried out on marine environments and while there has been an increase in work on understanding microplastic presence in rivers in recent years there still remains a gap in microplastic knowledge between freshwater and marine systems which needs addressing and which is understudied in an Irish context. Rivers, in particular, which are proposed to be the major source of plastic waste to marine environments deserve greater study. To date, this has been poorly investigated when compared to marine waters (Akdogan & Guven, 2019; Eekes-Medrano *et al*. 2015; Schmidt *et al*. 2017). Microplastics were recovered from every sample taken during the course of this study and found in sediment, water and *G. duebeni* in the rivers that flow into Dundalk Bay (SAC and SPA). Under descriptor 10 of the Marine Framework Strategy Directive for good environmental status, properties and quantities of marine litter should not cause harm to the coastal and marine environment. The ubiquitous presence of anthropogenic microplastics in the surface water of the rivers entering Dundalk Bay represents a threat to this environment. The increase in surface level concentrations closer to Dundalk Bay in 4 of 7 and 5 of 7 rivers for both sampling campaigns respectively indicates terrestrial inputs of microplastics, namely polyester and polyamide, into the riverine network that enters the bay. Given the shallow nature of Dundalk Bay the freshwater inputs may constitute a significant portion of microplastic pollution entering it. Microfibres were the dominant shape found in all matrices sampled while microbeads were documented with the rarest frequency. The contamination of *G. duebeni* at both upstream and downstream sites could lead to detrimental effects within individuals as well as representing a potential entry point of microplastics entering freshwater food webs for this area. The high levels of coloured microplastics present in the tissue of *G. duebeni* may indicate a preference for this type of material as opposed to transparent or white coloured microplastics potentially due to mistaking these items as food. For both sampling campaigns the location with the highest population pressure rendered the highest levels of microplastics in surface water which would indicate population pressure leads to increased microplastic burden in rivers for this catchment. Additionally, the 2nd most polluted site, the outflow location on the River Flurry may be due to the slope of the riparian zone and further studies should include the elevations between sampling sites of waterbodies. Sediment samples collected from the headwater and outflow riverbanks closely aligned in terms of microfibre constitution with surface water collected from the previous year highlighting it is potentially trapping this type of material. The behavior of the Irish public with respect to waste disposal (Omorodion, 2023) in combination with domestic wastewater infrastructure (EPA, 2022) may be a source of microplastic pollution for the rivers examined in this study. The use of the relatively cheap and safe potassium carbonate for extraction of microplastics from sediment in this study lends itself for increased accessibility for potential citizen scientist. Additionally, the use of a sieve stack down to 50µm led to the filtering of a large water volume (200L) and the retrieval of microplastics smaller than 330µm which can generally be missed when conducting surveying with more traditionally used nets or trawls. This study lends to the growing body of knowledge of microplastic prevalence in Irish freshwater environments (e.g., O'Connor *et al*. 2019; Murphy *et al*. 2022). This work is vital in order to develop a strong and credible baseline for microplastic pollution in the Irish environment to aid in future goverance and legislation which will likely be necessary to tackle this relatively new type of pollutant which is likely ubiquitous in the Irish environment with delitirous effects anticipated.

Chapter 4: Microplastic contamination of intertidal sediment and cockles (*Cerasastoderma edule*)

Stephen Kneel*, Caroline Gilleran Stephens[1], Alec Rolston[1], Ana M. Mendes[2], Liam Morrison[2], Suzanne Linnane[1]

Centre for Freshwater and Environmental Studies, Dundalk Institute of Technology, Dundalk, Co. Louth, A91 K584, Ireland[1]

Earth and Ocean Sciences, School of Natural Sciences and Ryan Institute, University of Galway, H91TK33, Ireland[2]

*Corresponding author: Stephen.kneel@dkit.ie

Highlights

- Microfibres were the dominant shape found in both sediment and cockles
- Lighter plastics PP and PE were only identified in cockles
- The high level of microfibres found indicate a need for better wastewater treatment facilities and legislation for these secondary microplastics

Graphical Abstract

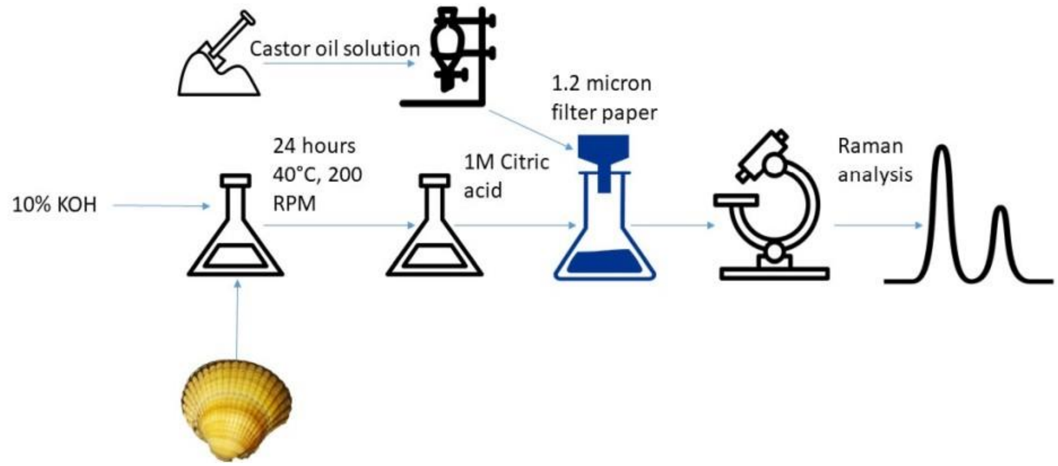


Figure 4-1: Workflow of current chapter from sample collection to polymer identification.

4.1 Abstract

Microplastic pollution represents a new threat to both marine environments and the species that reside within them. This study examined the temporal concentrations of microplastics found in the commercially and ecologically important bivalve, *Cerasastoderma edule* and the presence of microplastics in sediment taken from two beach locations within the intertidal environment of the Special Area of Conservation (SAC) and Special Protected Area (SPA) of Dundalk Bay, Ireland. A microplastic range of 1.55 ± 1.38 to 1.92 ± 1.00 g^{-1} in cockles was reported. Microfibres dominated the shapes of microplastics found in both sediment and cockles. A wider range of polymers were identified in cockles than in sediment in this study. Given the ubiquity of microfibres found amongst all matrices it is therefore important to investigate further potential sources that can have impacts on the marine environment.

Keywords: Microplastics, marine pollution, temporal, bivalves, sediment

4.2 Introduction

Microplastics or plastic particles <5mm, exist as either primary or secondary microplastics depending on their origin. They have been documented in a wide range of organisms and are prevalent in a huge range of environments including previously pristine environments such as deep-sea sediment and polar regions (Garza *et al*. 2023; Van Cauwenberghe *et al*. 2013; Peeken *et al*. 2018). Marine sediment can serve as both a source and a sink of microplastic pollution as well as regulating microplastic distribution in aquatic environments (Uddin *et al*. 2021; Jambeck *et al*. 2015; Woodall *et al*. 2014). The assessment of microplastic pollution falls under descriptor 10 of the Marine Strategy Framework Directive which states that anthropogenic litter should not cause harm to the coastal or marine environment and that quantities of litter entering these environments should be reducing overtime (MSFD 2008/56/EC). Microplastics can adsorb organic and inorganic pollutants from their surrounding environment. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organo-halogenated pesticides, nonylphenol and dioxins have been detected in plastic pellets from beaches worldwide (Avio *et al*. 2015; Ogata *et al*. 2009). If contaminated microplastics are ingested they may desorb these pollutants into organisms under the correct conditions (Bakir *et al*. 2014). Additionally, human pathogenic viruses have been documented on microplastics surviving up to 3 days in the environment (Moresco *et al*. 2022). Furthermore, exposure to pollutants that are known to adsorb to microplastics such as polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and oestrogenic compounds can delay the onset of oocyte maturation in cockles and reduce their fecundity (Matozzo *et al*. 2007; Timmermans *et al*. 1996; Bowmer *et al*. 1994).

Although microplastics have been recorded in many marine environments, they are particularly problematic in coastal locations due to the proximity of potential sources from the terrestrial environment and also tidal processes that can encourage their deposition and accumulation (Gray *et al*. 2018; Weinstein *et al*. 2016; Ryan *et al*. 2009). Microplastics have been documented in coastal environments in numerous recent studies and are found in varying concentrations worldwide, although limited studies have examined its presence in the Irish marine environment (e.g., Pagter *et al*.

2021; Martin *et al*. 2017). Intertidal locations in Ireland were noted to have a range of 0 to 553 particles per kilogram (Mendes *et al*. 2021). Across Europe, microplastics have been found in various concentrations in recent studies. Concentrations of microplastics of 53 ± 7.6 items per kilogram have been reported from the Black Sea which are similar to those recovered from the South-east of Spain, 64.06 ± 8.95 particles per kilogram (Terzi *et al*. 2022; Bayo *et al*. 2019). Two recent studies on microplastics in sediments from Europe reported concentrations of microplastics as 1.8 – 30.2 particles per kilogram in the Kiel fjord, Germany and as 0.02 – 1.71 microplastics per gram in Oslofjord, Norway, respectively (Schroder *et al*. 2021; Bronzo *et al*. 2021).

Microplastics have also been reported in many species of shellfish which may have implications for human health as some species can provide a direct route of microplastics into human diets (Li *et al*. 2021). Recently, microplastics have been documented in a wide variety of bivalve species including species of; mussels (Ding *et al*. 2021; Gedik and Eryasar, 2020; Wakkaf *et al*. 2020; Hermabessiere *et al*. 2019; Mathalon and Hill, 2014), clams (Baechler *et al*. 2020), oyster (Aung *et al*. 2022; Cho *et al*. 2019; Teng *et al*. 2019; Rochman *et al*. 2015; Van Cauwenberghe and Janssen, 2014) and scallops (Sui *et al*. 2020; Cho *et al.* 2019). While a lot of focus has been given to species of mussel (*Mytilus edulis,* in particular) very little work has been conducted on the common cockle *(Cerastoderma edule)* with just two prior studies examining the presence of microplastic in this species. One in populations on the French Channel coast (Hermabessiere *et al*. 2019) and a more recent one examining the population in Portuguese lagoons (Botelho *et al*. 2023).

Body condition indices have generally been the target of microplastic ecotoxicity studies on bivalves in the past, however there has been no noted impairment of these indices in several species including; *Scrobicularia plana* (Ribeiro *et al*. 2017), *Ennacula tenuis* and *Abra nitida* (Bour *et al*. 2018), *Mytilus edulis* (von Moos *et al*. 2012), *Crassostrea gigas* (Sussarellu *et al*. 2016), *Cerastoderma glaucum* or *Limecola balthica* (Urban-Malinga *et al*. 2021). Because of the little or no effects on body condition noted in ecotoxicology studies, bivalve body condition has been generalised as an insensitive marker of microplastic ecotoxicity (Bour *et al*. 2018). Microplastics however can impact the behaviour of bivalves living in sediment which in turn can lead to effects for benthic environments (Urban-Malinga *et al*. 2021). The near-surface dwelling species, *Cerastoderma glaucum* emerged less often and in lower numbers from sediment spiked with microplastics while the Baltic clam, *Limecola balthica* buried deeper in similarly treated sediment than in controls with no microplastic presence (Urban-Malinga *et al*. 2021). A similar reduction in surface emergence or increase in burial depth as displayed in the bivalves *Cerastoderma glaucum* and *Limecola balthica* (Urban-Malinga *et al.* 2021) when exposed to microplastic-contaminated sediment by *C. edule* an important species of bivalve found in Dundalk Bay could have negative consequences for benthic habitats given the many ecosystem roles that *C. edule* fulfils. Increased burial depth would lead to deeper fluidising of the seabed for harvesting of cockles by fishers in Dundalk Bay (currently 5cm) and the inability of seabirds in the area to feed effectively on them. The deeper fluidizing of the seabed as is the case currently for razor clam harvesting up to a depth of 0.25m can lead to a greater impact on benthic communities (Legare *et al*. 2020) An increase in the area fished to a greater depth would further jeopardise *Ensis. magnus*, *Pharus legume*, the spiny cockle (*Acanthocardia* spp), clams (*Venus* spp). *Chamelia* spp. and the endangered and long-lived Icelandic clam (*Arctica islandica*) in this SAC and SPA where abundances of these species is already low with richness declining since 2017 and *A. islandica* not recorded since 2018 (Shellfish Stocks and Fisheries Review, 2022).

This study represents a first look at temporal microplastic concentrations in a natural non-cultured population of commercially valuable bivalves in Irish fishing grounds. The common cockle, assessed in this study has potential as a biomonitor for microplastic pollution at the water-sediment interface in the marine environment for a number of reasons, including; its proximity to coastlines, its large geographical range and the fact it is easy to collect - generally residing in the top 5cm of marine sediment (Santos *et al*. 2022; Tyler-Walters, 2007). Furthermore, *C. edule* is monitored for *E. coli* presence by the Sea Fisheries Protection Authority (SFPA). While it is difficult to draw direct comparisons between studies given that tissue digestion and identification methods vary (Bom and Sá, 2021), microplastics presence is increasingly documented in marine species and further evidence of this is the presence of microplastics noted in rarer species from more remote regions (Li *et al*. 2021). Futhermore, microplastics were noted in a wide range of species inhabiting Dundalk Bay as evidenced in chapter 3 of this thesis.

A primary management goal is the maintenance of favourable conservation status of intertidal habitats such as Dundalk Bay where cockle fishing occurs in order to reduce the risk of recruitment failure in the future and ensure that conservation objectives for designated habitats and species are protected. The management plan for the cockle fishery in the SAC and SPA Dundalk Bay is crucial given their potential effects on designated habitats and birds (Shellfish Stocks and Fisheries Review, 2022). The pre-fishery survey estimate of cockle biomass in 2022 was 100 tonnes less than the preceding year and fishermen agreed not to take the Total Allowable Catch (TAC) of 608 tonnes and the fishery remained closed due to low market prices (Shellfish Stocks and Fisheries Review, 2022). The importance to establishing a baseline of microplastic presence in cockles in Dundalk Bay which can potentially be transferred to the seabirds feeding on them is of paramount importance. In northern fulmars and Cory's shearwaters the abundance of resident microbiota associated with healthy hosts decreased and conversely the abundance of microbes known to be involved in disease, antibiotic resistance, plastic degradation and zoonotic pathogens increased with the presence of microplastics in the gut (Fackelmann *et al*. 2023).

Previous studies carried out on assessing microplastic presence in *C. edule* did not examine microplastic contamination of sediment despite the fact that sediment-dwelling bivalves have been shown to possess higher concentrations of microplastics than water column-dwelling bivalves (Cho *et al*. 2021; Ding *et al*. 2021; Liu *et al*. 2021). Likely due to microplastics sinking as their density increases (Karkanorachaki *et al*. 2021).

The importance of a resilient cockle population in Dundalk Bay is threefold; 1) the economic value of the fishery, (€80,500 average per vessel in 2020), 2) supporting the seabird population that feeds upon them during winter, for example; overwintering oystercatchers (*Haematopus ostralegus*) in the absence of mussel beds require between an estimated 105-232kg cockle flesh per bird per winter (Ens *et al*. 2004) and 3) the ecosystem engineering roles it has in bioturbation, water-filtration and biodeposition (Carss *et al*. 2020). Due to the importance of cockles, both financially and ecologically to the habitat of Dundalk Bay, it is of paramount importance to assess the levels of microplastics in both this species and also in the sediment they reside in. Recently measures have been introduced to preserve some of Dundalk Bay and the species that reside therein with the banning of fishing using any kind of dredge, beam trawl or bottom otter trawl within a protected area which will hopefully lead to increased abundances and diversity of marine species in this restricted zone (Fig. 4-2; SFPA, 2023).

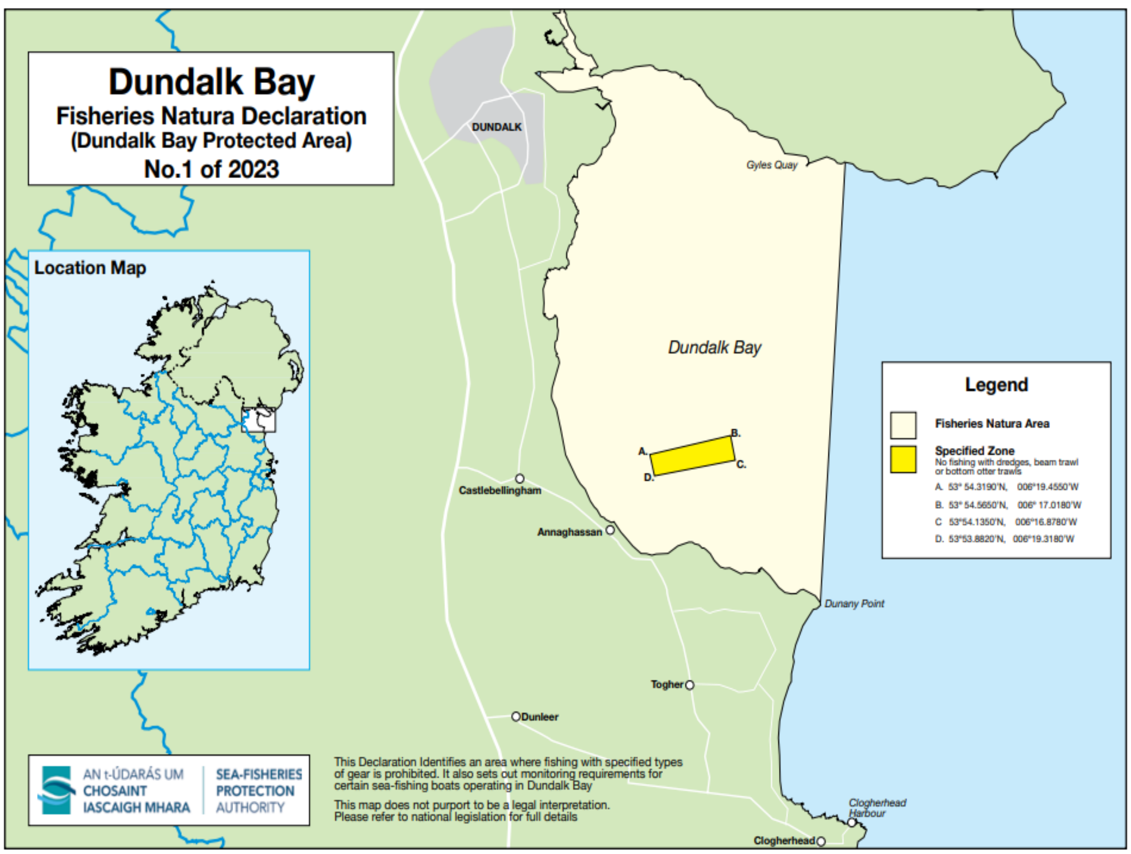


Figure 4-2: Dundalk Bay protected area highlighted in yellow (https://www.sfpa.ie/Who-We-Are/News/Details/sea-fisheries-protection-authority-publishes-reference-map).

The aims of this study were therefore; 1) to understand the quantities of microplastics present in cockles found in Dundalk Bay and to gain an insight into the types of polymers contaminating these species 2) to compare the quantities and polymer types of microplastics found in the sediment of two intertidal locations within the bay and 3) to examine the relatedness of these microplastics between the two examined matrices.

4.3 Study area

Dundalk Bay is a large shallow marine bay located on the east coast of Ireland, approximately 70km north of the capital of Dublin (Fig. 4-3). Dundalk Bay is classified a special area of conservation (SAC), special protection area (SPA) under the EU Birds and Habitats Directives and a Ramsar site (no. 834). As an SPA, approximately 23 species of bird are found there, while 6 unique habitats found in the bay qualify it as a SAC. Dundalk Bay is a classified Bivalve Mollusc Production Area and it has supported a commercial dredge cockle (*Cerastoderma edule*) fishery since 2001. The Dundalk Bay area is a prominent exporter of the common cockle which reaches markets for human consumption in Spain. Landings of cockle for Dundalk Bay for 2019 and 2020 were valued at €1,249,500 and €2,254,000 and weighed 595 and 1,127 tonnes respectively. A cockle permit is required to fish for cockles in Dundalk Bay with the number of vessels permits limited to 28 based on the historical fishing record of the vessel (Consultation on Dundalk Cockle Natura Permits Policy). Additionally, razor clams and in particular cockles are consumed by seabirds residing in the bay. As they are found closer to shore than razor clams, and up to 70% of the bay is exposed at low tide, cockles are an important source of food for wading birds residing there (Fig. 4-4). Dundalk Bay is internationally important for water birds, supporting over 1% of the Northwest European/East Atlantic flyway populations of the protected black-tailed godwit (*Limosa limosa*). (https://rsis.ramsar.org/ris/834). The shallow nature of Dundalk Bay is advantageous to cockle growth. Cockles found in the bay need to survive only one winter to reach commercial size while in most other areas growth rates are lower and cockles need to survive over two winters to reach commercial size (Shellfish stocks and fisheries review, 2021). Possible sources of microplastics to Dundalk Bay include; three wastewater treatment plants along its coast, the urban centres of Dundalk and Blackrock as well as agricultural sources and domestic settings further inland which can release microplastics that may be transported by rivers that drain the catchment area and empty into the bay. These microplastics can end up deposited in the sediment of the bay or be ingested by fauna residing there. Blackrock beach represents a site near a secondary water treatment plant and sees frequent beach users. Rockmarshall beach is a more secluded site however given the tidal movements of the Bay can represent a site where microplastic pollution is deposited. Sediment alone was collected from Blackrock beach in order to assess the differences in microplastic levels in sediment due to population pressures which are greater here than the more secluded Rockmarshall beach which hosts a cockle population.

Figure 4-3: Intertidal beach locations of this study, cockles were collected from Rockmarshall beach at the same site as sediment collection occurred.

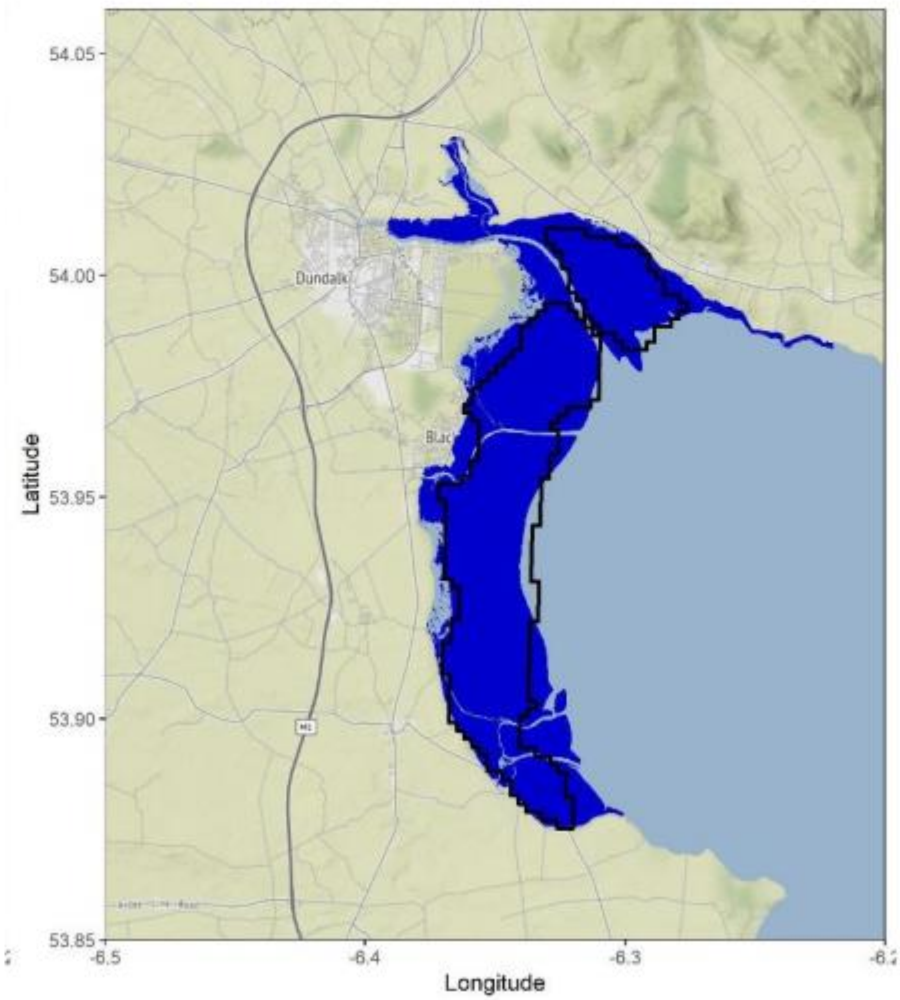


Figure 4-4: Estuaries, mudflats and sandflats of Dundalk Bay not covered by water at low tide in blue, the cockle fishery area outlined in black. (Marine Institute, 2021).

4.4 Study organism

The common cockle *(Cerastoderma edule)* is one of the main non-cultured bivalve species harvested around the coastlines of Western Europe where densities of populations can reach up to 10,000 individuals per m^2 (Carss *et al*. 2020; Tyler-Walters, 2007). It also has a very wide distribution, being found on the coasts of Northern Europe to those of West Africa (Hayward and Ryland, 1995). Cockles serve as a major food source for a variety of crustaceans, fishes and also wading-birds with the species-specific predation varying in accordance with the size of the cockles, bivalve larvae can even be ingested by bivalves including adult cockles through suspension feeding (André and Rosenberg, 1991). Cockles have been documented as a key resource supporting overwintering wader populations in species such as oystercatchers who require an estimated 105-232kg cockle flesh per bird per winter in the absence of mussel beds (main alternative food source), insufficient food availability leads to effects such as; reduced individual body condition, increased mortality and reduced population sizes (Ens *et al*. 2004; Verhulst *et al*. 2004). The importance of cockles in Dundalk Bay to sustaining the seabird population has been demonstrated by Albuixech-Martí (2021) where the highest percentage of *C. edule* DNA detected in seabird faeces samples from 6 intertidal sites nationwide were highest in the two sampled from Dundalk Bay.

The roles that bivalves play as ecosystem engineers is an important one, consisting of functions including bioturbation, water filtration and sediment modifications. Additionally, bivalves including cockles, perform biodepositional functions, filtering both organic and inorganic particles from the water column and transferring undigested particles to the sediment in faecal and pseudofaecal form (Urban-Malinga *et al*. 2021). Bivalves can therefore link microplastic pollution of the overlying water column and the bottom sediment of this environment, transporting these contaminants to the benthic environment, while can themselves be particularly susceptible to direct microplastic exposure given their feeding mechanisms (de Sá *et al*. 2018).

Besides serving as a food source for many species, including humans, the role of cockles in benthic habitats cannot be understated. The locomotion and burying activity of cockles lead to a continuous mixing of particulates in sediment, additionally the filtration and valve movements of the species increase pore water displacement and the exchange of solutes at the water-sediment interface (Mermillod-Blondin *et al*. 2005). Large-scale experiments also showed that cockles in high densities enhance sediment stability (sand) which is important to conserving and promoting the primary productivity of soft-bottomed intertidal ecosystems (Donadi *et al*. 2013). Cockles, in addition to increasing sandy sediment stability also enhance nutrient uptake efficient of the biofilm (Eriksson *et al*. 2017). The primary influence of cockles on biogeochemical dynamics in intertidal sediments is through their biodeposition and bioturbation activities (Rakotomalala *et al*. 2015; Mermillod-Blondin *et al*. 2004). Cockles filter organic material from the water column and produce faeces and psuedofaeces onto the sediment surface which can be sedimented and become fuel for the benthic microbial food web in deeper layers of substrate (Widdows and Navarro, 2007). Strikingly, the importance of the humble shellfish is commemorated in the place name Cocklehill and most recently, since 2018, in form of a Cockle Picker sculpture in Blackrock, Ireland by local artist Michéal McKeown (Fig. 4-5). Thus, the common cockle's importance to Dundalk Bay is threefold, having environmental, economic and social roles.

Figure 4-5: The cockle picker's statue overlooking Dundalk Bay from Blackrock beach. Photographed by Mark Duffy and appearing in the Irish Independent newspaper (23rd February 2019).

4.5 Materials and methods

4.5.1 Sampling

Cockles were collected for every month from February 2020 – January 2021. Fewer cockles were collected for the Spring (n = 28) and Summer (n = 28) due to Covid-19 restrictions than Autumn (n = 42) and Winter (n = 42). Cockles (n=140) were collected by hand raking from the intertidal area of Rockmarshall beach on the North side of

Dundalk Bay (54.002674N, -6.3000154W), placed in metal food containers, labelled and frozen at -20ºC. Sediments from the intertidal zones were collected from Rockmarshall beach(54.002674N, -6.3000154W) and Blackrock beach (53.961880N, -6.3651247W) during 2021 (Fig. 4-3). These coordinates represent the central point of the grids used to determine microplastic contamination on these beaches.

Sediment sampling was conducted similar to that described by Frias *et al*. (2018). Briefly, sediment was collected using a metal trowel (0<5cm) across 3 line transects (hereafter referred to as; upper-shore, middle-shore, lower-shore) located 50 metres from each other and 3 points were sampled across each transect line to give a total of 9 sampling points per beach in a square-like formation. Care was taken when digging up the sediment to avoid mixing deeper layers with the surface layer. Between sampling points the trowel was rinsed with Milli-Q water (0.22µm) to avoid cross-contamination. Samples were processed immediately upon returning to the laboratory.

4.5.2 Tissue digestion and microplastic extraction

Prior to microplastic extraction the biometric data of the cockles was recorded. Digestion was carried out using a protocol adapted from Thiele *et al*. (2019). Briefly, cockles were shucked, rinsed with Milli-Q water (0.22µm) and weighed before placing in 500ml Erlenmeyer flasks with 200ml of 10% potassium hydroxide (KOH). Tinfoil covered flasks were placed inside an incubator at 40ºC for 24 hours set to 250RPM, after which they were left to cool at room temperature. The samples were neutralised using 1M citric acid solution and vacuum-filtered onto clean filters (Whatman, 47mm diameter GF/C 1.2µm glass microfiber). Filters were then placed in closed petri dishes until further analysis was carried out.

4.5.3 Sediment separation

Sediment samples were oven dried at 40ºC for 24-48 hours depending on moisture content. Microplastic extraction from dried samples was carried out according to Mani *et al*. (2019). Briefly, 10g of sediment was collected using a spatula and placed in a 1L separation funnel and 100ml of Milli-Q water (0.22µm) and 10ml of castor oil were added. Separation funnels were then shaken by hand for 1 minute and the inner walls of the funnel were rinsed with Milli-Q water (0.22µm) to ensure all material was returned to the bottom. The funnel was left to settle for 15 minutes after which the underlying sediment layer was filtered from the separation funnel and set aside. The middle layer of water and upper layer of castor oil were vacuum-filtered onto filter papers (Whatman, 47mm diameter GF/C 1.2µm glass microfiber). While the separation funnel was being emptied the walls were rinsed with pre-filtered (Whatman, 47mm diameter GF/C 1.2µm glass microfiber) 70% ethanol to ensure all material was removed. The inner walls of the top part of the vacuum filtration unit were then rinsed with 70% ethanol to help to remove any castor oil left on top of the filter paper which may obscure subsequent microplastic visualisation. For each of the 9 points where samples were collected per beach, 3 x 10g samples were analysed.

4.5.4 Grain size analysis

Samples were oven-dried at 60℃ and 100g of dry sediment was weighed and placed in a 6-sieve stack (2mm (gravel), 1mm (very coarse sand), 500µm (coarse sand), 250µm (medium sand), 125µm (fine sand) and 63µm (very fine sand) and a receiver for sediments <63µm. Sediment was classified using an adaptation of the Wentworth grain size classification (Frias *et al*. 2018).

4.5.5 Visual examination and Raman analysis

Filter papers containing both digested cockle tissue and material extracted from the marine beach sediment was observed under an Olympus SZX7 microscope. Microplastics were counted, measured and their respective colour and shape was recorded. Microplastic colours were classified as; transparent/white, blue, red, green, black, multi-coloured or other. Microplastics were measured along their largest diameter. Finally, microplastics were designated a shape profile as fibre, fragment, film or bead and characterised based on their response to metal tweezers (plastics should not break under stress but should flex or bend) (Keene and Turner, 2023). Microscopic analysis was carried out by an individual researcher to allow consistent comparisons between shapes and colours. Microplastics from the sediment and cockle samples were selected at random for characterisation and polymer identification using Raman Spectroscopy. The Raman Spectrometer was equipped with a 600 groove mm^{-1} diffraction grating, a confocal optical system, a Peltier-cooled CCD detector, and an Olympus BX41 microscope (Ó Briain *et al*. 2020; Loughlin *et al*. 2021) and spectra were obtained at a range of 100–3500 cm^{-1} using a 532nm laser. Spectra obtained when analysing particles extracted from cockles and sediment were compared to a spectral reference library (KnowItAll, Bio-Rad), an in-house extension of the library with additional spectra from environmental plastics collected from the intertidal zone and known virgin polymer types (purchased from CARAT GmbH, Bocholt, Germany) (Mendes *et al*. 2021). In addition, SLoPP and SLoPP-E libraries (Munno *et al*. 2020) were employed, and the 'fingerprint' region of each spectrum was used to identify the polymer type. The websites 'Open Specy' (Cowger *et al*. 2021) (https://openanalysis.org/openspecy/) was also used to verify polymer type for spectra captured via Raman in addition to PublicSpectra (https://publicspectra.com/SpectralSearch). Furthermore, the Infrared & Raman Users Group (http://www.irug.org/search-spectral-database) was also consulted.

4.5.6 Statistical analysis

Data was tested for normality using Anderson-Darling test which determined that both MPs g^{-1} and MPs ind^{-1} were non-parametric. MPs g^{-1} values were successfully transformed using Johnson transformation ($P = 0.807$) while MPs ind^{-1} values were not. One-way Analysis of Variance (ANOVA) tests and Tukey post-hoc analysis were used to determine statistically significant differences between average microplastics per gram wet weight (MPs g^{-1}). The Kruskall-Wallis test was carried out on the non-parametric determining that significant differences existed between the seasons in terms of microplastics ind^{-1}. Mann-Whitney tests were then carried out for each potential set of seasons to examine which were statistically different from each other. Regression analysis was carried out on the average microplastics of cockles per individual and gram wet weight and the seawater temperature. The non-parametric Spearman's correlation coefficient was calculated to evaluate correlations between carried out on the total cockle group (n = 140) to determine if there was a statistical relationship between the weight of cockles and their microplastic content. Mann-Whitney tests were carried out to assess microplastic amounts in contamination controls and those recovered from cockles as prescribed by Dawson *et al*. (2023). The level of significance used for all tests was 0.05. Anderson-Darling tests revealed that distribution of microplastics in sediment followed normal distribution for both beach locations and one-way ANOVA tests was used to determine if the values of microplastics from any points used to collect samples on each beach were significantly different to each other. Statistical analysis was carried out using Microsoft Excel 2016 and Minitab Statistical Software (version Minitab® 21.1.1 (64-bit) which was also used for the creation of figures.

4.5.7 Quality control measures

To ensure good laboratory practises when analysing samples for microplastics protocols for mitigating microplastic contamination were adhered to where applicable following Hermsen *et al*. (2018). When conducting labwork 100% cotton lab coats were worn at all times. All work conducted on samples including the aforementioned vacuum-filtrations were carried out in a "clean room" which was specifically used for microplastic work exclusively by a lone operator. Equipment such as; tweezers, sieves and glassware were covered in tinfoil when not in use, additionally the vacuum-filtration unit was covered in tinfoil when filtering digested cockle or sediment samples. While cockles were being dissected, a petri dish containing a filter paper was placed adjacent to quantify airborne contamination. Procedural blanks were carried out at the same time as the digestion of cockle tissue and sediment separation. All equipment used for sample collection and analysis were triple-rinsed using Milli-Q water (0.22µm) before use, and glassware was left to dry upside down to avoid airborne particles settling on them. These measures were necessary to avoid microplastic contamination from particles that may be present in the air and settle on equipment leading to an overestimation of microplastic numbers. Fibrous microplastics which are present in outdoor and indoor air have the potential to settle on equipment which may lead to an overestimation of the amount present in a sample matrix (Gasperi *et al*. 2018). All solutions used in this study were vacuum-filtered through clean filter papers before use with samples (Whatman, 47mm diameter GF/C 1.2µm glass microfiber). Despite following these procedures, it is often impossible to completely mitigate microplastic contamination and it is therefore important to quantify it. For each beach site, 6 oil separation blanks were carried out in order to account for contamination while for each group of cockles (n=14) a solution blank was also examined. As with numerous other microplastic studies (e.g., Baechler *et al*. 2020; Rochman *et al*. 2019; Davidson and Dudas, 2016) contamination results from the blanks are displayed (Appendix B: Table 8-6, Table 8-7) rather than subtracted from environmental results which is ill-advisable given the diversity available in microplastic shape, size, colour and composition (Dawson *et al*. 2023). These are intended to indicate the range of possible contamination levels present introduced from laboratory procedures. Mann-Whitney tests carried out for microplastic contamination in terms of both MPs g^{-1} and MPs Ind^{-1} found that amounts present in control blanks were significantly lower than those present in environmental samples (P = 0.000, P = 0.000) respectively. The mean microplastic contamination for Rockmarshall and Blackrock beaches accounted for 9.1% and 13.3% of the mean microplastics in 10g samples for each beach.

4.5.8 Biometric data

Biometric data of cockles analysed in this study were as follows: wet weight of cockles ranged from 0.9-7g, (mean: 2.81g ± 1.04), length ranged from 2-4cm (mean: 2.86cm ± 0.38cm) and the width ranged from 2.3-4.2cm (mean: 3.19cm ± 0.42). The differences between seasons are displayed below (Table 4-1). The cockles in this study were on average above the legal landing shell size width of 17mm and the effective landing minimum size of 22mm which is used to separate Irish from UK fisheries cockles on the market (Fishery Natura Plan for cockle (*Cerastoderma edule*) in Dundalk Bay, 2021-2025).

Table 4-1: Biometric data of cockles for each study season.

Season	Gram wet weight	Length	Width
Spring	2.71±0.71	3.04±0.32	3.33±0.31
Summer	2.62±0.85	2.83±0.33	3.16±0.35
Autumn	3.59±1.04	3.02±0.33	3.41±0.37
Winter	2.21±0.88	2.60±0.35	2.90±0.42

4.6 Results and discussion

4.6.1 Microplastics in cockles

A total of 697 microplastics were visually isolated and sorted from 140 bivalves in this study. Just two cockles analysed during this study were free of microplastics while the vast majority had microplastics present in their tissue resulting in a 98.6% contamination rate of the cockles analysed. When assessing mean microplastics per gram wet weight (MPs g^{-1}) this value ranged from the lowest in winter 1.55 ± 1.38 MPs g^{-1} to a highest value in spring 1.82 ± 0.99 MPs g^{-1} for cockles sampled, two outliers were also noted amongst cockles sampled in Autumn and Winter however the reasons for these is unclear (Fig. 4-6).

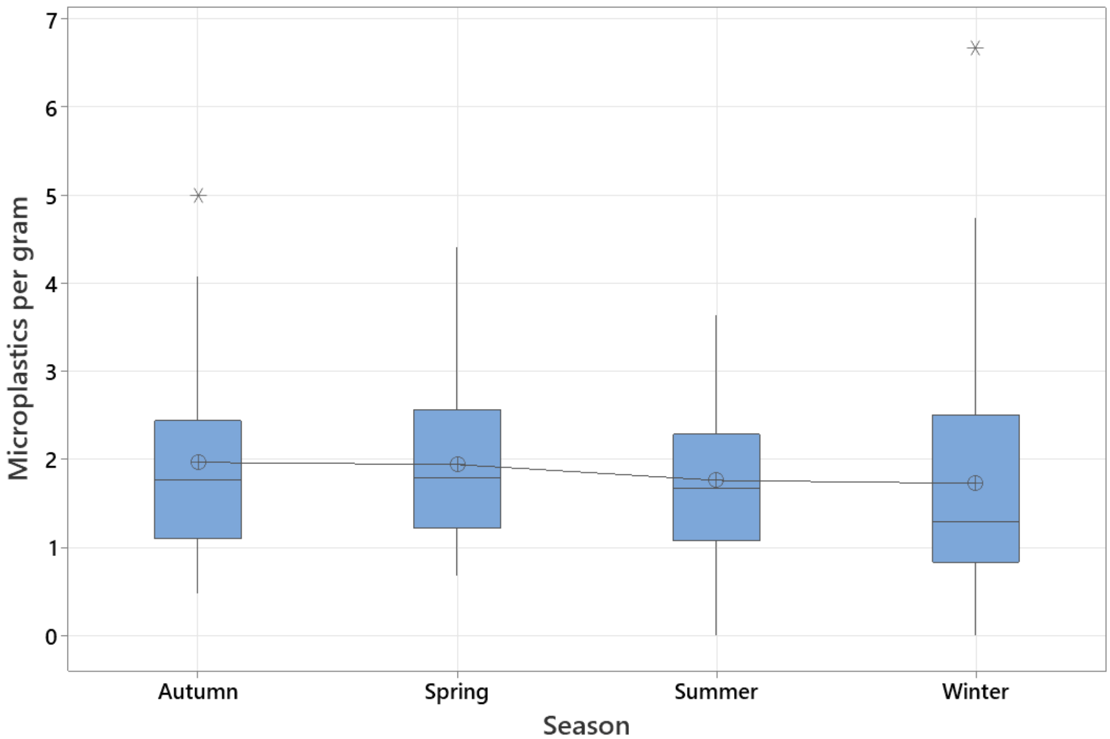


Figure 4-6: Seasonal microplastic contamination per gram wet weight of cockles. Asterisks represent outliers. Circles with cross interior represent the mean. Solid horizontal lines represent the median. Blue rectangles represent the interquartile range Q1 – Q3 and whiskers represent the range.

One-way ANOVA test at the 95% confidence interval showed that average MPs g^{-1} of cockles were not significantly different between seasons (P=0.283), although the median and mean values for cockles harvested in winter were lower than the other seasons. Cockles examined from a Portuguese lagoon displayed significant difference in their microplastic concentrations between seasons and similarly had lowest reported concentrations for winter (Botelho *et al*. 2023). Similarly, four species of bivalves studied by Ding *et al*. (2021) displayed no seasonal variation either in terms of microplastics g^{-1} of microplastics ind^{-1}. Cockles analysed in this study had generally higher microplastic concentrations than reported in the same species along the French Channel coasts by Hermabessiere *et al*. (2019), which contained 0.94 ± 0.31 items g^{-1} wet weight, however, cockles in that study were collected from areas with a small human population and low anthropogenic pressures. This range fell within reported median values for *C. edule* sampled from a Portuguese lagoon of 0.83 items g^{-1} and

5.1 items g^{-1} and also mussels (*M. galloprovincialis*) in the same study 0.77 items g^{-1} in January to 4.3 items g^{-1} in October (Botelho *et al*. 2023).

Average microplastics per cockle (MPs ind^{-1}) ranged from 3.43 ± 2.47 particles per cockle in Winter to 6.90 ± 3.68 particles per cockle in autumn (Fig. 4-7). Kruskall-Wallis testing revealed statistically significant differences between seasons in terms of MPs ind^{-1} ($P = 0.000$). Mann-Whitney tests carried out between seasons revealed that the only seasons that did not significantly differ between medians were spring and summer ($P=0.422$) but every other comparison displayed significant differences between seasons with regard to this parameter. In real-world terms, however, Autumn was the season that had biological significance compared to the other seasons studied and the mean and median for this season was much greater than the others studied (Fig. 4-7). One cockle sampled in Autumn reported the highest volume of microplastics per individual with 17 revovered, another outlier was noted in Spring cockles where 13 were recovered in one cockle (Fig. 4-7). Cockles were on average larger for this season than those collected in the other seasons which may help to attribute to this difference, especially as MPs g^{-1} did not display significant differences between seasons. The higher abundances of microplastics in cockles collected in autumn for this study highlights the potential entry to the human diet that these can have as the cockle fishery closes on the first of November annually when it has been preceded by 14 weeks open and cockles are harvested for human consumption in this period (autumn).

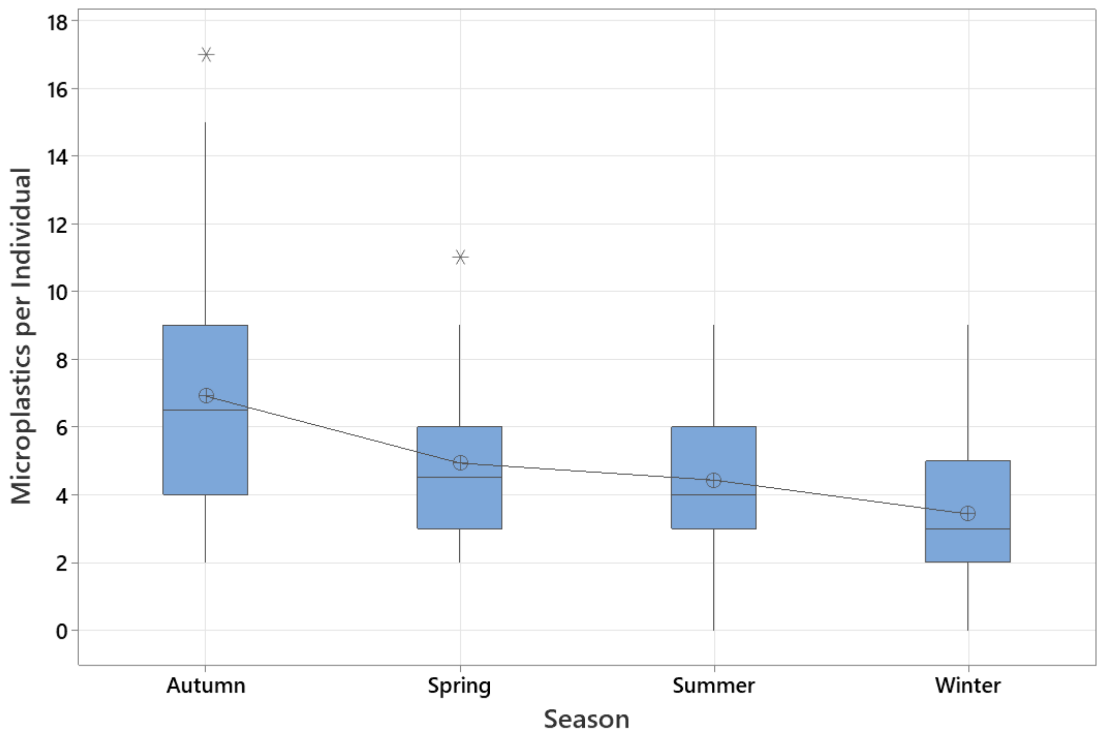


Figure 4-7: Seasonal microplastic contamination per individual for cockles. Asterisks represent outliers. Circles with cross interior represent the mean. Solid horizontal lines represent the median. Blue rectangles represent the interquartile range Q1 – Q3 and whiskers represent the range.

These values are similar to some other reported microplastic levels noted for different species of bivalve such as; 1.5-7.6 reported for *M. edulis* collected from Chinese coasts and 6.9 ± 3.84 reported for *M. bilineata* from Southeast India (Li *et al*. 2016; Patterson *et al*. 2019). In general, however, the upper values of individual contamination range found in cockles in this study (autumn) is higher than reported individual contamination of bivalves than in other studies. Samples of the same species taken along the French Coast also had lower levels of microplastics than those reported in this current study with reported values of 2.46 ± 1.16 MP ind^{-1} (Hermabessiere *et al*. 2019) while Botelho *et al*. (2023) did not provide this data.

Microfibres were the most commonly noted microplastic shape and accounted for 69.6% (n=485) of those recovered. Other shapes were identified with lower frequency; fragments (18.2%, n = 127), films (11.2%, n = 78) and finally beads were seen with the lowest frequency accounting for 1.01% (n = 7) of the total recovered. Fibre proportions were similar between seasons ranging from 67.5% of the total recovered in autumn to 72.5% of the total recovered in spring which could indicate a stable input of this sort of microplastic into this marine environment. Cockles from locations along the coasts of France had comparatively lower microfibre levels than those analysed in the current study and ranged from 2.4% to 45.5% per site per site (Hermabessiere *et al*. 2019). Similarly, a lower proportion of fibres were documented in cockles collected from a Portuguese lagoon for both winter and summer seasons studied with fibres accounting for 50% and 38% of those recovered (Botelho *et al*. 2023). The proximity of the WWTP located on the Castletown River may be a source of microfibres seen in this study as its effluent location is located just 3.5km from the location cockles were collected from for this study in addition to the freshwater rivers which contain microplastics in their surface waters as displayed in chapter 3 of this thesis.

While the lowest proportion of microfibres were noted in cockles collected in autumn and summer (which had the highest average sea temperatures), there was only a small difference in the comparative proportions made up of fibres between seasons. There are several possible reasons for this occurrence. Typically, during hotter months, there is less need for domestic tumble-drying of clothes, the condensate of which is a source of microfibres entering wastewater treatment facilities (Gaylarde *et al*. 2021). Additionally, hotter sunnier periods increase the fragmentation of plastics into microplastics, this is especially true for beaches where macroplastic items are exposed to UV radiation and high oxygen availability leading to increased photo-oxidation (Andrady, 2015). Macroplastic items have been observed by the author in close proximity when collecting cockles throughout this study. Furthermore, reduced precipitation during the hotter periods inhibits the potential washing of microplastics from terrestrial sources into rivers which typically have lower flow rates at these times, however, this reduction was not as pronounced due to weather conditions in the country for summer and autumn. For the year 2020 an unseasonably strong Jetstream dominated the weather in Ireland for summer which brought above average rainfall for the season, and near to, or below average temperatures (Met Éireann, 2020).

Although microfibre concentrations in cockles varied between seasons, microfibres were still the dominant shape found every season, with fragments and films representing a smaller proportion (Fig. 4-8). This is in agreement with research on marine species as a whole, as per Mizraji *et al.* (2017) microfibres constitute the dominant shape category of microplastics consumed by marine fishes, crustaceans and bivalves while microplastic fragments, foams, and films represent a smaller proportion of microplastics found in these species (Jabeen *et al*. 2017).

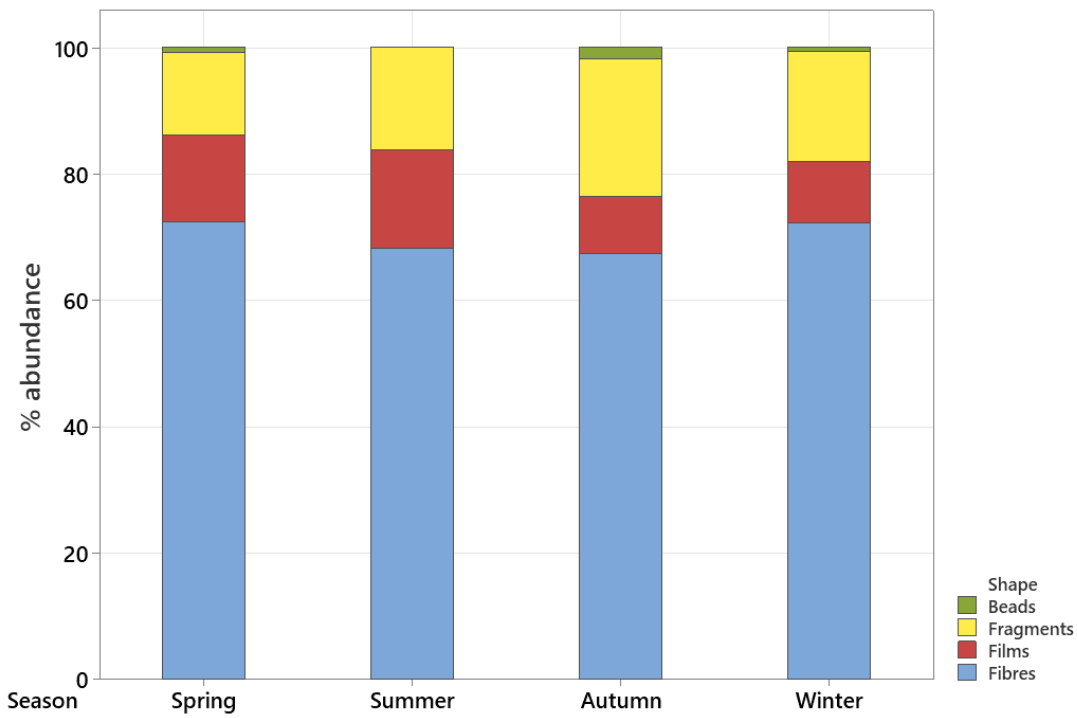


Figure 4-8: Shape composition of microplastics recovered from cockles for different seasons.

Fibre contamination of cockles in this study was also similar to those found in oysters harvested from New South Wales which possessed fibre concentrations in the range 43% - 80% (Jahan *et al*. 2019; Li *et al*. 2018). The high proportion of microfibres found in cockles despite the fact they possess gills that can trap particles prior to transport to the mouth of the organism and ingestion may suggest, that just as microfibres when orientated correctly can pass through the meshes of sieves (Walkinshaw *et al*. 2022), a similar phenomenon occurs with the gills of cockles.

Zoonotic protozoan parasites (*Toxoplasma gondii*, *Cryptosporidium parvum*, and *Giardia enterica)* that can infect and harm humans and marine mammals were shown to have a greater ability to associate with microfibres than with microbeads (Zhang *et al*. 2022). The World Health Organisation recognises these pathogens as underestimated causes of illness from shellfish consumption additionally they are persistent in the marine environment. Furthermore, faeces of seabirds feeding on cockles found in Dundalk Bay have been shown to contain *Vibrio* bacteria common to both sediment and cockles (Albuixech-Martí *et al*. 2021) thus highlighting the potential for microplastics to act as vectors of disease transmission for this area.

It is implied that the amounts of microplastics in mussels increase with their growth (Berglund *et al*. 2019), as cockles are also filter feeders this relationship between cockle wet weight and microplastic burden was examined. Based on the Spearman correlation test there was a weak but statistically significant relationship between cockle weight and microplastic abundance (correlation = 0.438, P <0.000) (Fig. 4-9).

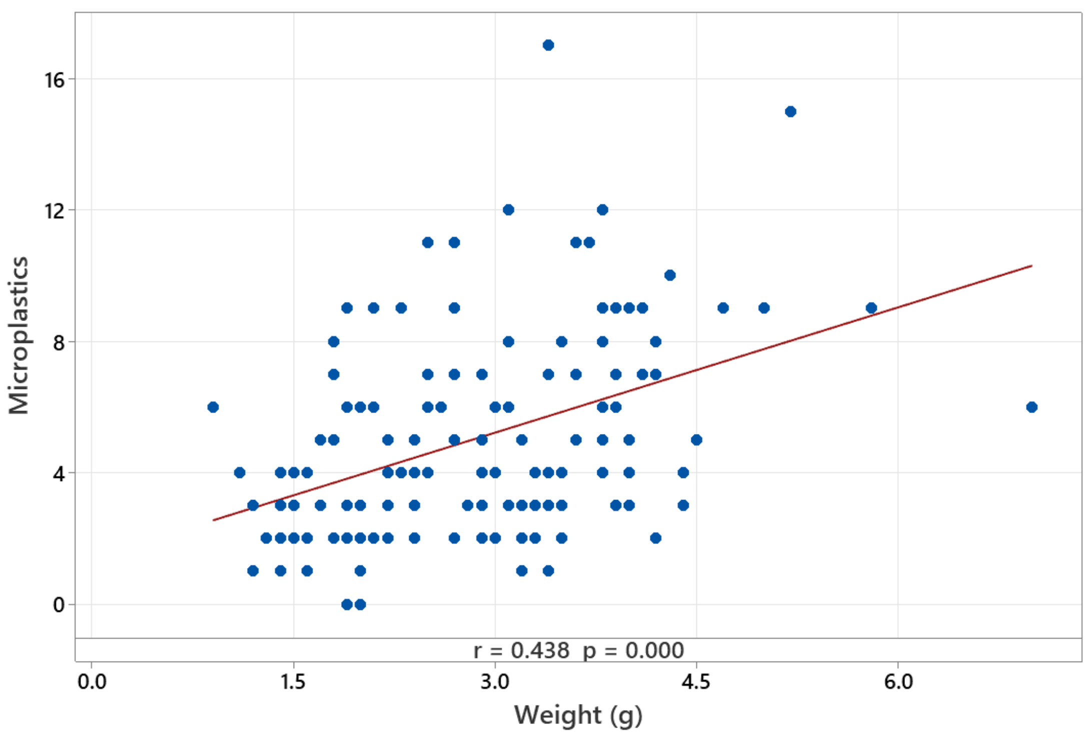


Figure 4-9: Relationship between microplastic burden and weight of cockle.

Filtration rates for seawater generally increase with cockle body size (increased gill surface area), however factors such as; food availability, temperature and physiological (mainly reproductive) conditions can also play a role (Smaal *et al*. 1997; Iglesias *et al*. 1996). Cockle filtration rates tend to be highest in the temperature range of 8-20°C (Brock and Kofoed, 1987), especially for the spring season in order to provide the energy necessary for gonad development (Newell and Bayne, 1980). Filtration rates, however, strongly reduce in water temperatures less than 8°C even with the abundant availability of food (Smaal *et al*. 1997).

The highest amounts of microplastics were found in cockles harvested in autumn both in terms of MPs g^{-1} and MP ind^{-1} when the mean water temperature in Dundalk Bay was 14.3°C which was the highest average seasonal temperature (Sea Temperature Info, 2022). The fact that there were no significant differences in microplastic concentrations in terms of MPs g^{-1} between seasons may indicate that microplastics in this species are potentially escaping egestion and may have relatively long residual times. More likely, however, is that due to the shallow nature of Dundalk Bay (which lends to the productivity of the cockle fishery) that even in colder month's cockle filtration rates increase when submerged in a few inches of water which is rapidly warmed by solar radiation during the day. Furthermore, the similar microplastic concentrations between seasons may be due to a combination of environmental factors for the marine environment of Dundalk Bay. In warmer seasons, the temperature increase leads to increased filtration rate of cockles which could increase the exposure to microplastics present in the water while in colder seasons the filtration rate decreases but the freshwater inputs increase as the flow rates increase for rivers that enter Dundalk Bay (EPA Hydronet, 2022) which can lead to an increase in terrestrially based microplastics entering the Bay. This may result in a similar microplastic exposure level even as filtration rates decrease. Furthermore, the possibility of import of microplastics from nearby coastal waters should not be excluded (Lozano-Hernández *et al*. 2021) as several studies in coastal environs have displayed the import of suspended particles from the sea during periods of low river discharges (Dias *et al*. 2007; Falcão and Vale, 2003; Vale, 1990).

In bivalves collected in a Portuguese lagoon, median concentration of microplastics in cockles were statistically lower in winter than summer and was attributed to the temperature decrease in winter potentially triggering a lower filtration rate, resulting in lower concentrations in the whole-soft body tissues of organisms (Botelho *et al*. 2023). Given that sea temperature can be a factor governing cockle filtration rates, this was examined as a predictor for microplastic concentrations and numbers in individuals using regression analysis. However, this was not deemed to have an overall strong impact on the relationship for either accounting for 33.3% of the variation in MPs ind^{-1} and 16.7% of the variation for MPs g^{-1}.

Although cockles do not select food based on sight and therefore colour unlike some species of fish (de Sá *et al*. 2015), it can be important to record the colours of microplastics, as it can be an indication of their age. Song *et al*. (2017) documented that polystyrene that underwent long-term exposure of UV irradiation changed the colour from white to yellow and became fragile. Transparent / white microplastics primarily fibrous in nature were the most commonly recovered in the current study, accounting for 50.1% (n=349) of total microplastics recovered and were the predominant colour found for every season studied. Blue microplastics were the next most commonly recovered, and accounted for 22.8% (n=159). Black (n=62, 8.9%), red (n=34, 4.9%), green (n=18, 2.6%), multi-coloured (n=11, 1.6%) and other colours (n=64, 9.2%) were also recovered (Fig. 4-10).

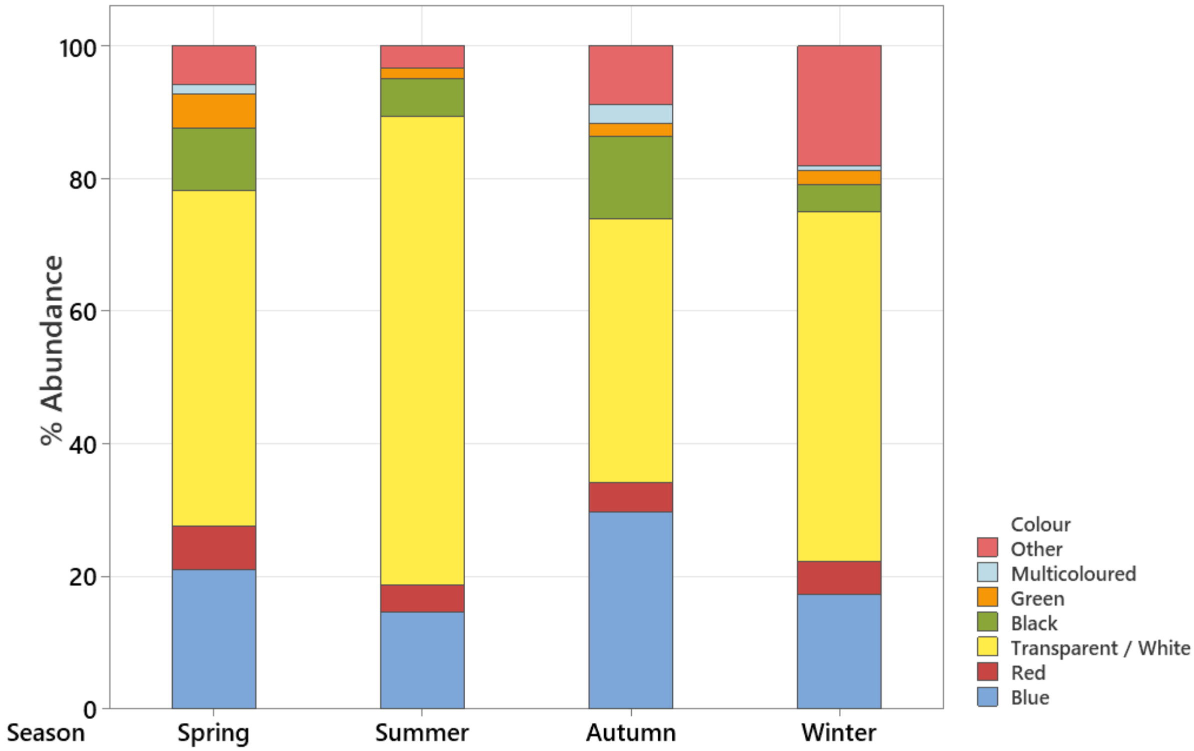


Figure 4-10: Seasonal microplastics colour composition found in cockles.

Documenting the size profile of microplastics is important as it can demonstrate their bioavailability to organisms that encounter them. In this current study the majority of microplastics were <1mm in size for each season. The proportions within these smaller categorises (<1mm) differed between seasons (Fig. 4-11). These findings are in line with the findings of Joyce *et al*. (2022) who found that microplastics <1 mm was the most common size recorded in the benthic Dublin Bay prawn, *Nephrops norvegicus*. Microplastics in the size category 1 – 5mm accounted for 38.3% of the total 697 microplastics recovered from bivalves respectively. According to the microplastic size classification method developed by the National Oceanic and Atmospheric Administration, 50% of microplastics found in aquatic systems are between 500µm – 1mm, 29.8% of microplastics are between 1 and 2.5mm and 17.6% of microplastics are between 2.5 and 5.0mm (Revel *et al*. 2018).

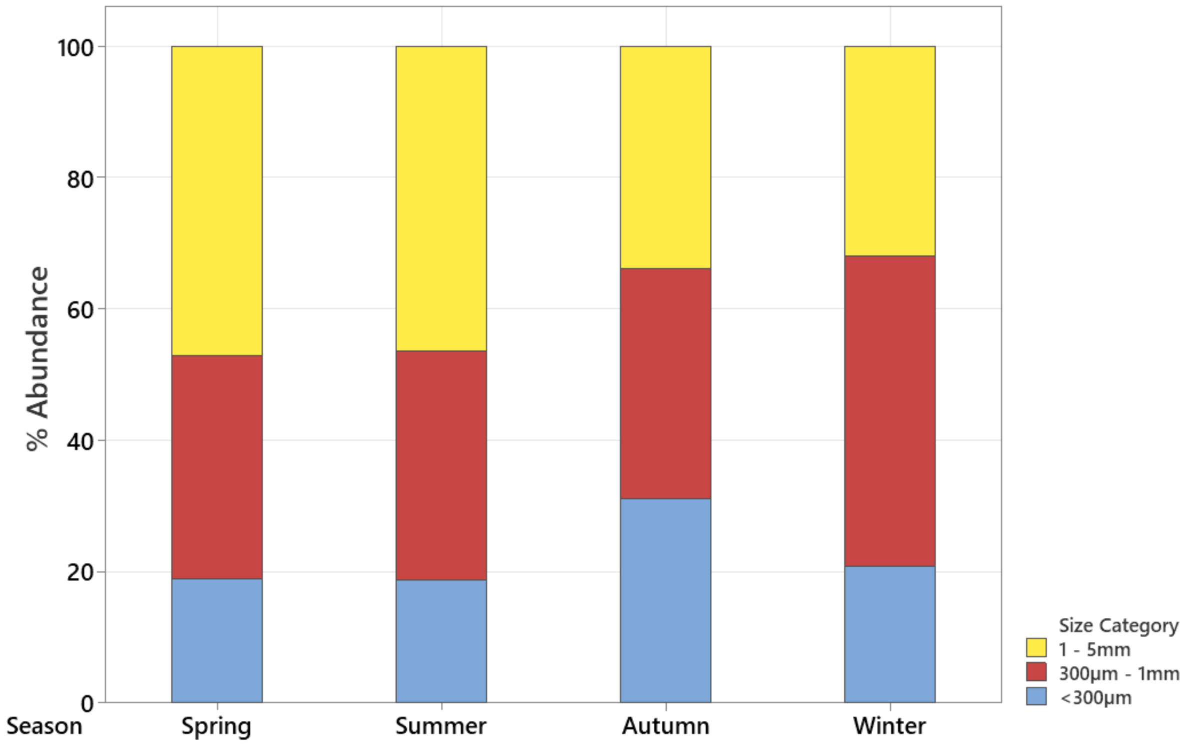


Figure 4-11: Seasonal microplastic size composition found in cockles.

Microplastics recovered from cockles in this current study were generally larger than those found in cockles and mussels on the channel coasts of France where 0.9% were larger than 500µm (Hermabessiere *et al*. 2019). However, larger particles have been documented in this species previously. Karlsson *et al*. 2003 observed the uptake of cellulose between 60 and 500µm in this species and also sand grains of up to 600µm present in the intestines. Additionally, Kristensen (1957) found that adults of the species inhale bivalve larvae up to the size of 900µm. It has also been noted that for particles with a high aspect ratio such as fibres ingestion is less constrained in bivalves provided that one dimension is within the size that can be ingested (Ward *et al*. 2019). For example, when microspheres and microfibres were delivered simultaneously to oysters and mussels both species rejected significantly higher proportions of the 1000µm diameter spheres than 1075µm long fibres (Ward *et al*. 2019). Larger microplastics (> 25µm) are likely to have relatively short transit times in bivalves and will primarily be concentrated in biodeposits (pseudofeces and faeces) (Ringwood, 2021). This, however, means that cockles can act as transporters of microplastics in the water column to marine sediment and may have effects on other species found there.

4.6.2 Microplastics in marine beach sediment

For each of the sampling points (n=9) per beach, the average microplastic content was taken from the 3 x 10g samples that were analysed. The raw data is displayed in boxplots for both beach locations and their respective nine sampling points below and shows the variability between replicates (Fig. 4-12, Fig. 4-13). The average number of microplastics per beach was determined by taking the average number of microplastics from the 3 x 10g samples analysed per point adding these now nine average 10g values together and averaging them before extrapolating to MPs kg^{-1} dry weight.

From Rockmarshall beach, the maximum number of microplastics found in a 10g replicate was 20 while for Blackrock beach the maximum number was 39 microplastics. This is much lower than the study by Liebezeit and Dubaish (2012) which recorded a maximum of 496 microplastics in one 10g sediment sample. Mathalon and Hill (2014) reported microfibre concentrations ranging from 20 – 80 / 10g from Halifax Harbour beach, while bays and beaches of Huatulco, Mexico had values ranging from 0 – 69 microplastics per 10 g dry weight of sediment (Retama *et al*. 2016). One-way ANOVA testing found that there was no significant difference between the mean microplastic concentrations for sampling points on each separate beach, (Rockmarshall beach; $P = 0.29$) (Blackrock beach; $P = 0.48$). While there were no significant differences in microplastic numbers between sampling points on the two beaches there was high variability between replicates taken. This underlines the potential of under- or overestimating microplastics in the environment in instances were replicates are not taken which has previously been noted in another study on microplastics in sediment of the Irish marine environment (Pagter *et al*. 2020a).

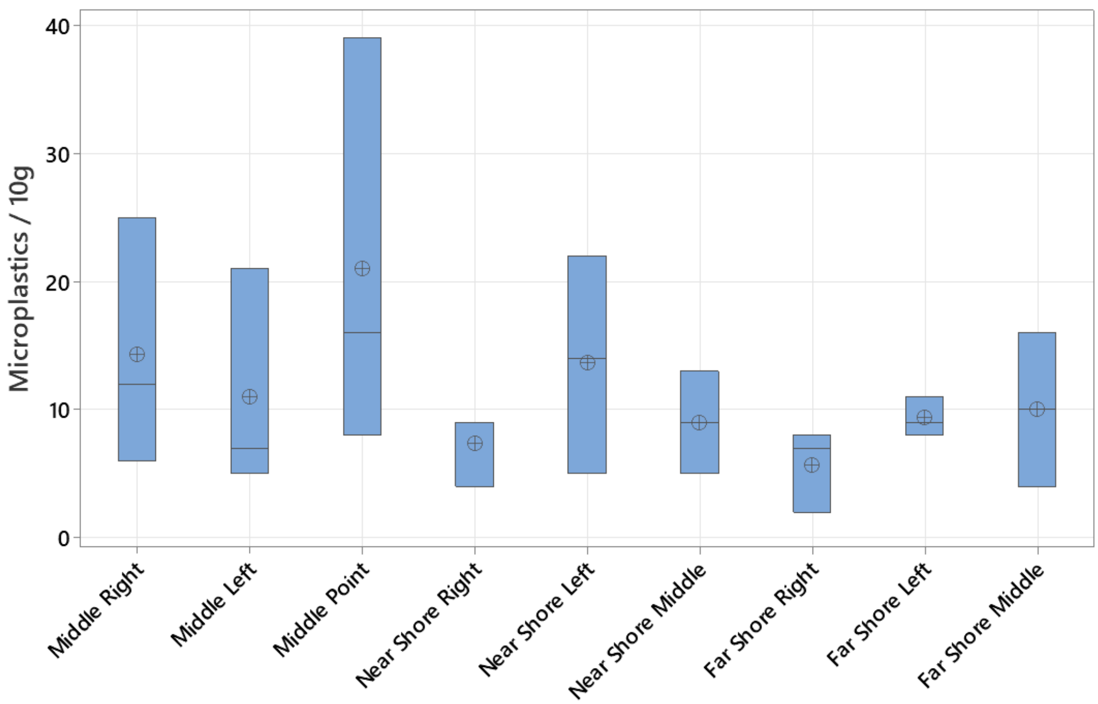


Figure 4-12: Microplastic levels of sediment from 9 sampling points on Blackrock beach. Circles with cross interior represent the mean. Solid horizontal lines represent the median. Blue rectangles represent the range.

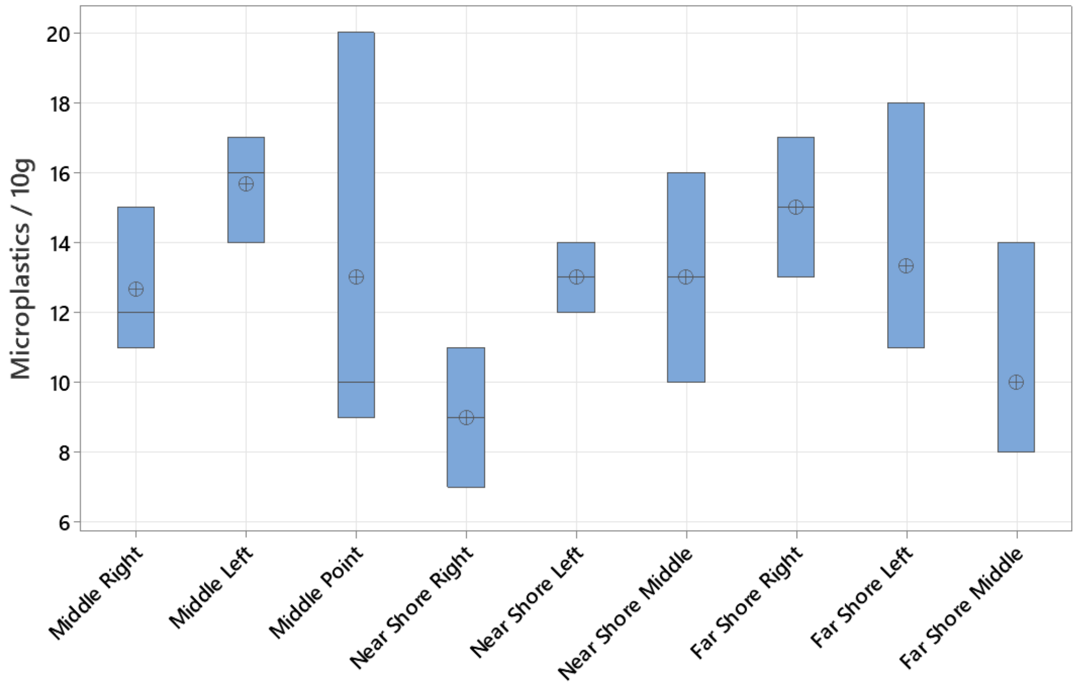


Figure 4-13: Microplastic levels of sediment from 9 sampling points on Rockmarshall beach. Circles with cross interior represent the mean. Solid horizontal lines represent the median. Blue rectangles represent the range.

Microplastics were recovered from every replicate sample taken from all locations. Generally speaking, this is in line with most studies on beach or intertidal locations, however some studies on shallow coastal environments have reported at least one replicate where no microplastics were detected (e.g., Ferreira *et al*. 2020; Sandre *et al*. 2019; Laglbauer *et al*. 2014). In total 344 microplastics were recovered from 270g of beach sediment (1274 MPs Kg^{-1} dw) from Rockmarshall beach on the North coast of Dundalk Bay whilst 304 microplastics were recovered from beach sediment (1125 MP kg^{-1} dw) from Blackrock Beach inner coastline. Microplastics were recovered in a variety of shapes, sizes and colours at both study sites. Microfibres were the most commonly observed shape of particle recovered at both locations. accounting for 83.1% and 82.5% of the total microplastics recovered from Rockmarshall and Blackrock beach respectively (Fig. 4-14). The next most common particle shape recovered at both beaches were fragments (Rockmarshall; 13.9%; Blackrock; 12.5%) followed by films (Rockmarshall; 2.6%; Blackrock; 4.6%) and finally microbeads accounted for 0.003% of total microplastics recovered at both locations. The predominance of microfibres found in sediment in this study was similar to results observed in other studies on Irish marine sediment (Mendes at al. 2021; Martin *et al*. 2017; Joyce *et al*. 2022) and in the cockles analysed for microplastic contamination as part of this study. A review of microplastic contamination of marine coastal environments by Harris (2020) found that all but 3 of 13 studies that reported shape composition of recovered microplastics from beaches had microfibre compositions above 70%, and that the median number of microplastics that are fibres from 12 studies on shallow coastal environments was 60%. The presence of two nearby WWTPS to both sampling locations may help to explain the high proportions of microfibres found at both beach locations as they are less likely to be trapped in WWTPs than other shapes (Ben-David *et al*. 2021). Although the WWTP plant located next to Blackrock beach caters for a smaller population equivalent (PE) than the WWTP located nearer to Rockmarshall beach it must be noted that this is a secondary treatment facility compared to the WWTP located nearer to Rockmarshall beach which has tertiary treatment but caters for a larger PE. The differences in microplastics removal efficiency between these facilities has been noted previously with tertiarly treatment plants more effective at capturing microplastics (Magni *et al*. 2019; Lyare *et al*. 2020; Tang and Haibarata, 2021; Edo *et al*. 2020).

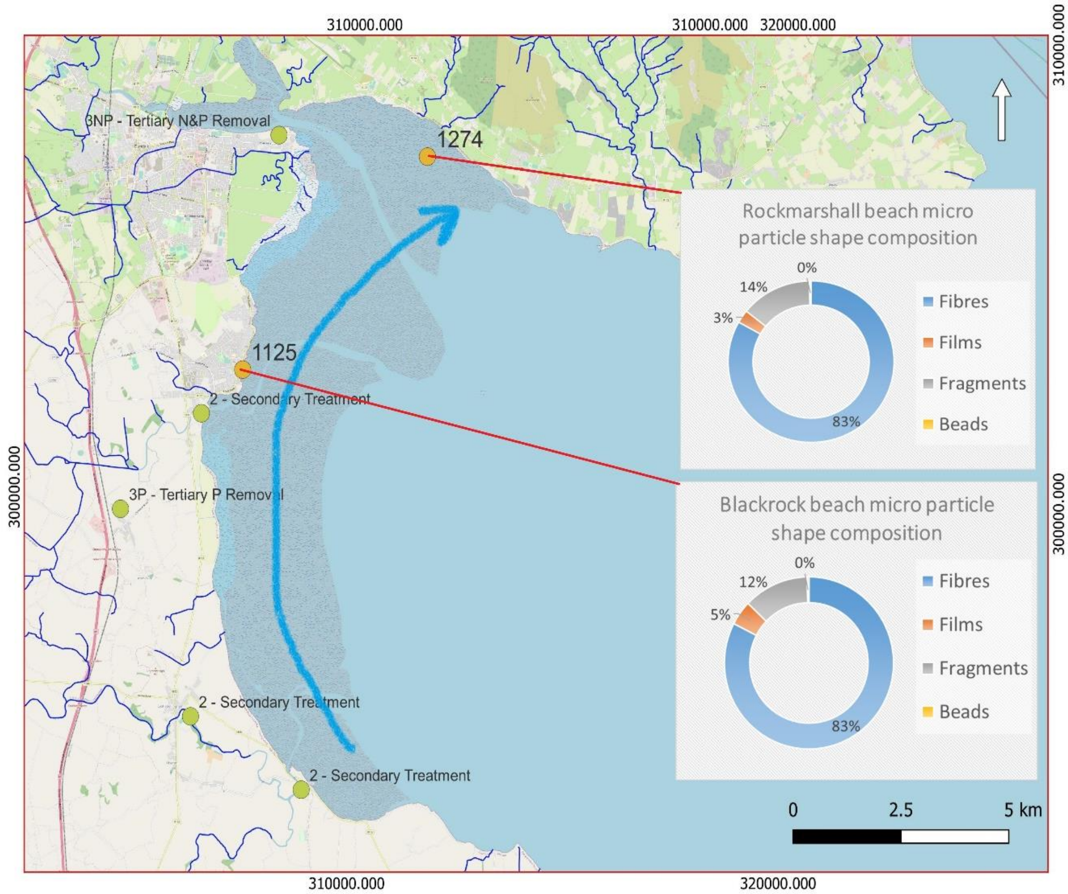


Figure 4-14: Concentrations (MPs Kg^{-1}) and shape composition of microplastics recovered from sediment of Dundalk Bay and proximity to wastewater treatment plants and rivers that enter the bay. The bay fills from the South when the tide comes in, the direction of which is indicated with a blue arrow.

The majority of microplastics recovered from both beach sites were transparent / white in colour and accounted for 53.7% (n=185) and 49.3% (n=150) of the total recovered Rockmarshall and Blackrock sites respectively, material blue and black in colour were the next most abundant with 57 (16.5%) and 43 (12.5%) items recovered at Rockmarshall. Red (n=28, 8.1%), green (n=10, 2.9%), multi-coloured (n=5, 1.4%) and other colours (n=16, 4.6%) made up the colour profile of the remaining portion of recovered items from Rockmarshall beach. A similar pattern in colour composition was seen in microplastics from Blackrock beach. Blue was the next most abundant colour found there (n=63, 20.7%), followed by; red and black (both; n=25, 8.2%), other (n=26, 8.5%), green (n=13, 4.3%) and multi-coloured (n=2, 0.6%). A similar colour profile of microplastics has been documented previously in marine sediments with white, black, blue and transparent particles occurring frequently (Díaz-Jaramillo *et al*. 2021; Baptista *et al*. 2019; Martin *et al*. 2017). As noted by Zhao *et al*. (2022) the influence of colour should also be considered when assessing the ecological risk and toxicity of plastics in the environment. It must be remembered that the colour of a plastic particle cannot easily be used to deduce its type or origin as microplastics may change colour to transparent or yellow due to weathering or sample processing and also have originally been transparent in nature (Zhang *et al*. 2020; Su *et al*. 2018). Colour composition of sediment sampled from the same location as cockles (Rockmarshall beach) was similar to that found in cockles. Colour composition was also similar between both clams and sediment studied in a Southern Philippine Estuary with black and blue particles dominating (Bonifacio *et al*. 2022).

It is important to categorise the size distribution of reported microplastics as not only does it enable discussion about potential bioavailability, but it also enables understanding of their potential transport and dispersal in the marine environment via currents and waves in relation to their hydraulic equivalence to natural sediment particles (Harris, 2020). Microplastics larger than 1mm made up the majority found at Blackrock beach (54.9%) while they made up the minority (33.1%) found in sediment at Rockmarshall beach. One possible reason for this difference is the very close proximity of the WWTP located at Blackrock to the sampling location meaning that microplastics are readily deposited in the upper layers of sediment here without undergoing subsequent breakdown in the natural environment to form smaller microplastics, while the beach location at Rockmarshall is further from both WWTPs on the coast of Dundalk Bay, potentially receiving more degraded and therefore smaller ‘older’ microplastics. Further research is needed to test this hypothesis though and the transport mechanisms for differently sized microplastics differ, moving either as suspended-load or bedload which can further fragment them (Harris *et al*. 2020).

4.6.3 Polymer analysis of microplastics isolated from cockles

A subsample of suspected microplastics (n = 110, 15.7%) was randomly selected from cockles for polymer identification using Raman spectroscopy and matches were identified for 85 of these. The most commonly identified were Polyamide (PA), Polypropylene (PP), and polyesters (Fig. 4-15). Unspecified polymers are discussed in chapter 2 (section 2.9.2) of this thesis. Material of natural origin, namely, cellulose made up 9% of the total identified using Raman spectroscopy. Some examples of identified polymers are displayed below (Fig. 4-16).

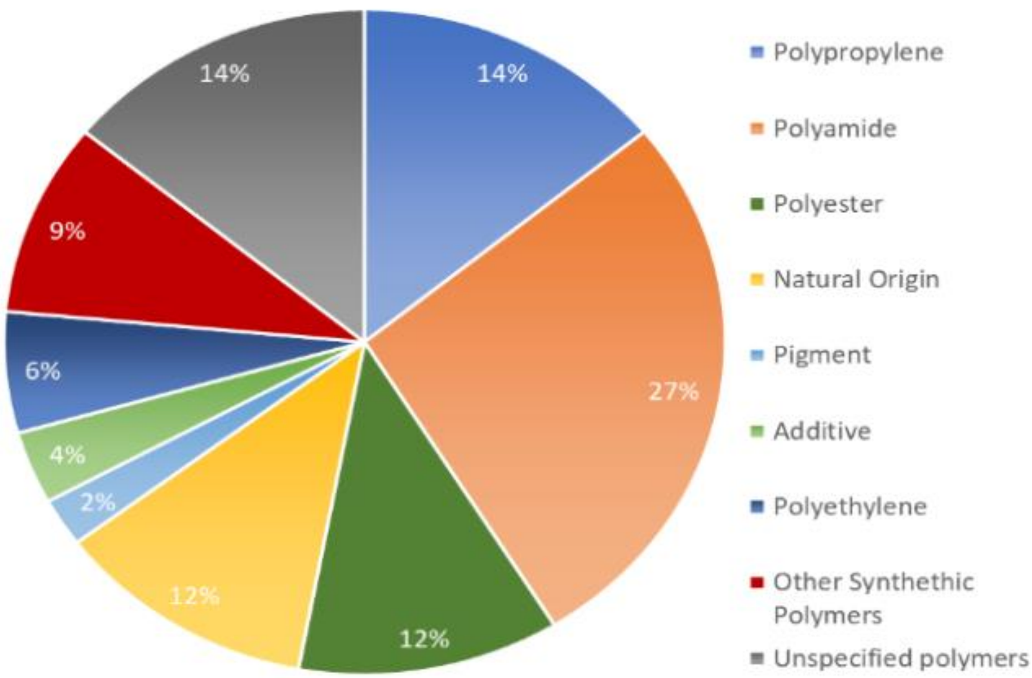


Figure 4-15: Polymer composition of microplastics recovered from tissue of *C. edule.*

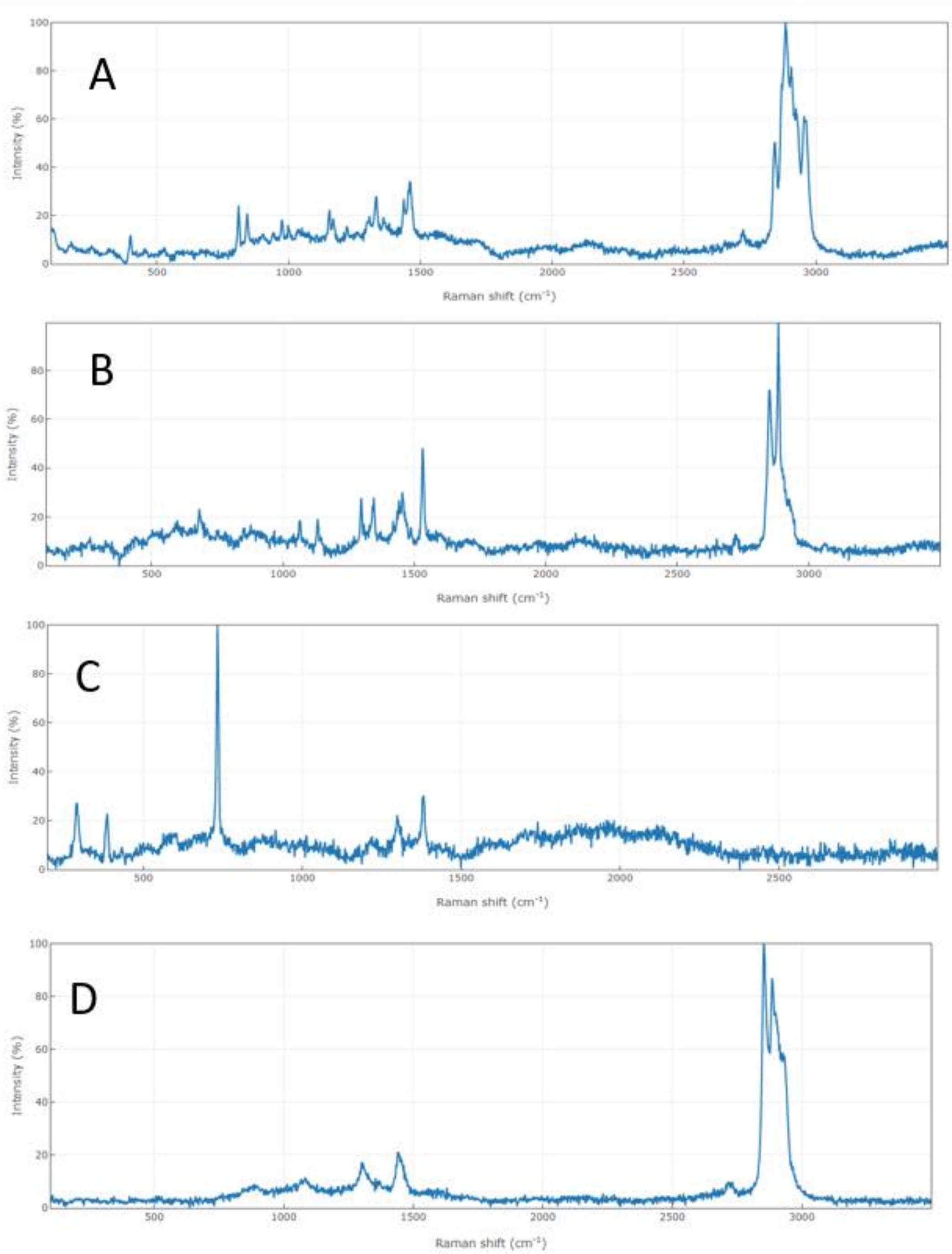


Figure 4-16: Examples of polymers identified from cockles. A: polypropylene, B: polyethylene, C: perfluoroalkoxy alkane, D: poly(ethylene-co-1-hexene).

4.6.4 Polymer analysis of microplastics isolated from marine beach sediment

A subsample of suspected microplastics (n = 144, 22.2%) was selected at random from those recovered from beach sediments and positive matches were obtained for 112 of these (Fig. 4-17). The most commonly noted polymers were PA, unspecified polymers and polyesters which made up 77% of those recovered. There was a wider range of polymers recovered from the sediment from Blackrock beach with 9 identified (PA, PS, Polyethylene terephthalate (PET), Polyvinyl chloride (PVC), ethylene vinyl alcohol (EVOH), Polycyclohexylenedimethylene terephthalate (PCT), Polystyrene-poly(ethylene propylene)block-polystyrene (SEP) and a copolymer of poly(butylene terephthalate and poly(tetramethylene glycol) (PBT-PTMG). Cellulose made up the majority of material of natural origin found in both cockles and beach sediment. Examples of some polymers identified in sediment are displayed below. (Fig. 4-18).

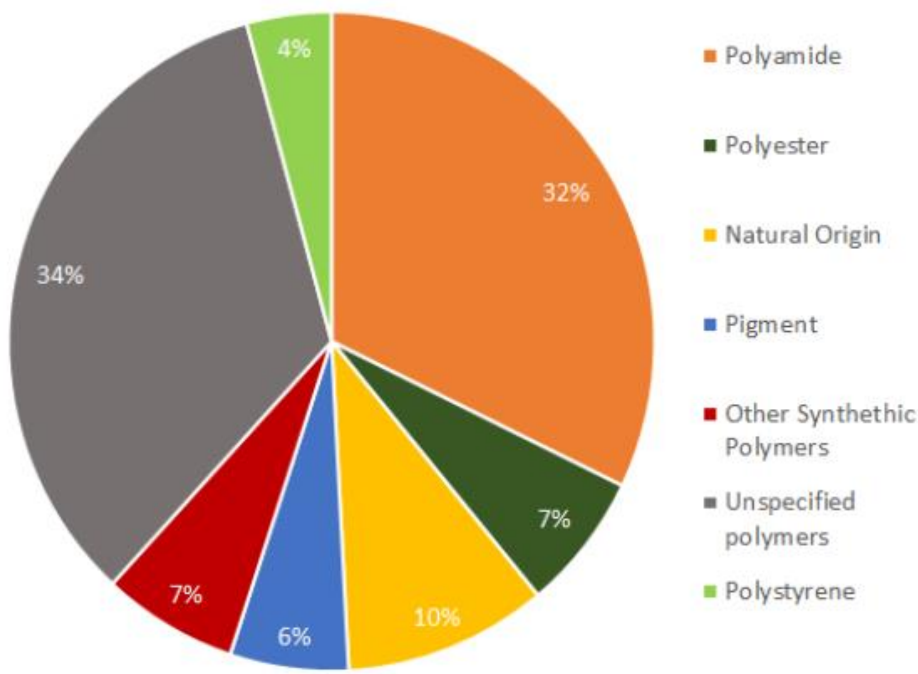


Figure 4-17: Polymer composition of microplastics recovered from intertidal beach sediment.

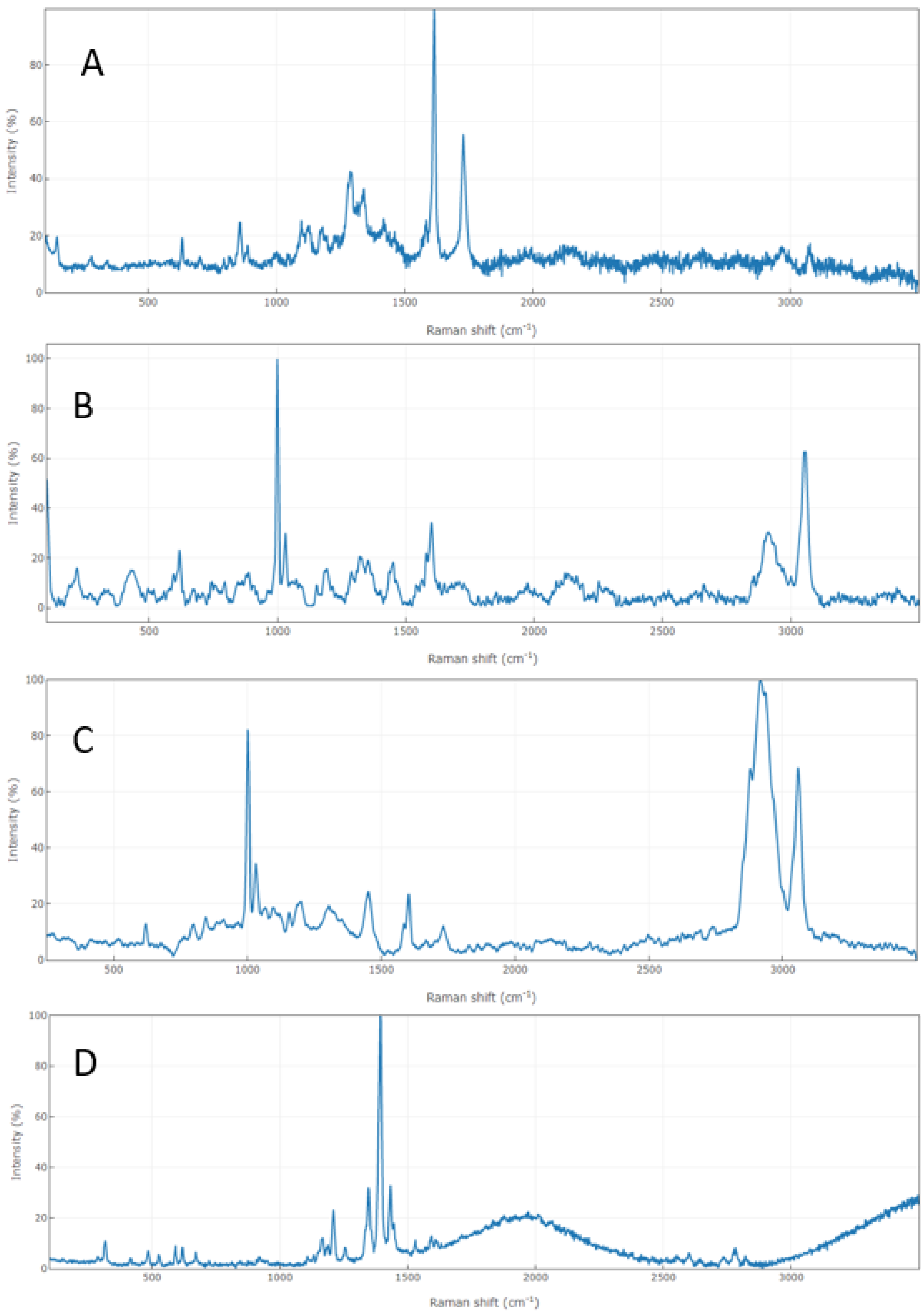


Figure 4-18: Examples of polymers identified from sediment. A: polyester, B; polystyrene, C; polystyrene-poly(ethylene propylene)block-polystyrene, D; pigment violet 23 (dioxazine).

4.6.5 Comparison of identified polymers between matrices

While polyethylene and polypropylene were both recovered from cockle tissue it was not recovered from the sediment of either beach and is likely due to their densities. These polymers are the two most commonly produced plastics accounting for approximately 49% of plastics demand distribution by resin type in 2019 with many widespread uses such as; packaging, agricultural film, piping, houseware and automotive parts (Plastics Europe, 2020).

However, this is not a totally unprecedented occurrence, Martin *et al*. (2017) noted that PA was the dominant polymer recovered from marine sediment from the Irish continental shelf while polypropylene made up just 3% of those identified. Additionally, the presence of PE or PP was not detected in subtidal sediments taken from the West of Ireland (Pagter *et al*. 2020a). While both of these plastics are commonly used in fishing equipment as components of nets, ropes or floats they have a density lower than that of seawater which accounts for their abundance in ocean surfaces (Morét-Ferguson *et al*. 2010) while being less dense than PA and PVC which are also used in fishing gear (Lusher *et al*. 2017; Andrady, 2011). PA and polyesters were also frequently recovered from the bottom dwelling Dublin Bay prawn *Nephrops norvegicus* and sediment from Irish fishing grounds in a recent study (Joyce *et al*. 2022).

Their presence in cockle tissue may be due to the feeding mechanism involved with this species filtering seawater for nutrients in and ingesting these buoyant polymers as the tide comes in without them settling down to the sediment upper layer in great numbers. Cockles may thus give a representation of microplastic presence at the water-sediment interface in shallow marine environments.

While PA may have fishing-based sources they are also used in the production of fabrics (Coyle *et al*. 2020). PA in the form of nylon 6 and 6,6 in combination with polyester account for the largest global share of synthetic textile microfibres (Castelvetro *et al*. 2021). The fact that the vast majority of microplastics recovered from both cockles and sediment were fibrous in nature which leads to the likelihood that they stemmed from sources such as WTTP effluent or being washed from their sludges when applied to land as biosolids (Corradini *et al*. 2019). A large portion of both inflow and outflow microplastics to WWTPs were found to consist of textile microplastics (Long *et al*. 2019, Murphy *et al*. 2016). Polyester and PA are expected to sink faster due to their high density and are mostly found in intertidal and subtidal sediments (Botelho *et al*. 2023). Fibrous microplastics were also the dominant shape found in surface waters studied in chapter 3 of this thesis.

There was a wider range of polymers recovered identified in the tissue of cockles than in the sediment of Dundalk Bay. PP, PE, PCT, PA, PE, PET, Poly(butylene terephthalate)-poly(tetramethylene glycol) (PBT-PTMG), Poly(ethylene-co-hexene), an ethylene copolymer, Poly(ethylene-vinyl acetate), acrylic rubber were all identified. Furthermore, titanium dioxide which is used in a broad range of plastics was detected as well perfluoroalkyl alkane, a forever chemical. This wide range of recovered polymers indicate that *C. edule* may be a suitable bioindicator species to be collected in conjunction with sediment samples able to filter both buoyant and sinking plastics. However, alone it is unlikely to be effective as a biomonitor for these buoyant plastics and it may be useful to collect a species that resides in the water body and has greater access to more buoyant polymers such as PE and PP such as the mussel, *M. galloprovincialis* as demonstrated by Botelho *et al*. (2023) in conjunction with *C. edule*.

4.6.6 Grain size analysis

The sand grains from both beach locations varied in composition. At Rockmarshall, the sediment consisted of gravel (0.02%), very coarse sand (0.08%), coarse sand (0.52%), medium sand (35.63%), fine sand (60.05%), very fine sand (3.34%) and silt, clay or colloids (0.36%). Sediment from Blackrock beach was finer in composition and consisted of gravel (0.01%), very coarse sand (0.08%), coarse sand (0.42%), medium sand (4.03%), fine sand (62.87%), very fine sand (30.72%) and silt, clay or colloids (1.87%). The relationship between sediment grain size and microplastic load has been examined in several studies.

Mendes *et al*. (2021) established an association between finer sediment particles and greater contamination of microplastics in Irish marine sediment while a lack of relationship displayed between microplastic load and sediment grain size has been noted in other studies such as; Mathalon and Hill (2014), Dodson *et al*. (2020), Alomar *et al*. (2016) and Browne *et al*. (2010). In this current study, however, Rockmarshall beach which had coarser overall sediment content than Blackrock beach had a greater microplastic burden but this difference was not significant. Additionally, more microplastics in the larger 1 – 5mm size range were recovered from Blackrock beach (54.33%) than from Rockmarshall beach (33.22%). For beaches in this very shallow and flat bay it is therefore possible that the nearby presence of wastewater treatment plants and the tidal movement in the bay are governing factors for microplastics presence in sediment, however, further study examining microplastic transport in this area is needed to verify this.

4.7 Conclusion

In summation, there were many advantages to the process undertaken to assess microplastics in cockles and sediment of Dundalk Bay. Since the assessment of the microplastic content in cockles was total body content (no organ specific digestion was performed) in combination with the rapid freezing of the bivalve samples (no-depuration) gives relevant environmental results as predators (crabs, seabirds etc.) do not target specific organs or parts of cockles when preying upon them and can themselves be exposed to the total microplastic content of cockles. Predation of contaminated prey items is one of the primary routes of microplastic exposure (Nelms *et al*. 2018). The use of castor oil for microplastic extraction from sediment in this study has numerous benefits. It has environmentally friendly disposal, a non-toxic nature enabling speedier use in lab and is cheap when compared to other density separation salts such as $ZnCl_2$ or NaI and allowed the recovery of denser polymers such as PVC and PET as noted by (Mani *et al*. 2019). These factors allow its use in citizen science projects which are key in order to establish effective monitoring for microplastics on both a local and national scale.

The lack of a strong linear relationship between cockle weight and microplastic burden and biological differences between seasons indicates that cockles in this marine environment ingest and likely egest microplastics persistently and microplastic presence is likely a transient value and a reflection of surrounding microplastic levels, they could however, serve as vectors for disease infecting this important shellfish.

Ecologically important cockle beds in Dundalk Bay are a prominent source of food for wading birds and it is likely they are ingesting microplastics with the potential for transferring potential pathogenic bacteria to these species before being egested themselves to other areas. This network of pathogen / microplastic transport between cockles and seabirds for this area requires further study. Furthermore, populations of cockles have been subject to mass mortality events in the past resulting from a variety of causes, including: harsh winter conditions and exposure, overfishing, predation, overfishing, failed recruitment, pollution and diseases (Ducrotoy *et al*. 1989). Due to the vulnerability of cockles to a wide range of threats, the contamination of cockles in Dundalk Bay by microplastics represents a relatively new threat to this population of vital shellfish that have an economic, ecological and historical importance to this SPA and SAC.

The results of this study show that cockles found in Dundalk Bay are contaminated with microplastics and can lead to predators being exposed to microplastic contamination. They can also exacerbate the contamination of other organisms found in the sediment of the bay through the egestion of microplastics they filter from the water column. To comprehend in full the impact that microplastics can have on intertidal or shallow ecosystems, large-scale mesocosm studies may be necessary. While the potential sources of microplastics to this environment are numerous and likely include but are not limited to; fishing activities, littering, riverine inputs, and urban pressures and the effluent of WWTPs on the coastline of Dundalk Bay as microfibres were the most frequently noted shape for both cockles and sediment. Microfibres dominated the microplastic shape morphology present in surface water samples present in rivers entering Dundalk Bay reported in chapter 3 of this thesis thus contributing to the contamination of the marine environment.

Microfibres constituted the greatest proportion of microplastics found in cockles for every season studied as part of this study, with a range of 67.5 to 72.5% of total abundances and the majority of microplastics found in sediment at both beaches which indicates that cockles have suitability in reflecting environmental microplastics types for this area. Recently, the potentially pathogenic group, *Vibrio*, was detected in *C. edule*, sediment and bird faecal samples where the oystercatcher, *Haematopus ostralegus* and other waders were observed to be feeding on cockles at intertidal areas around the Irish coast (Albuixech-Martí *et al*. 2021). Microplastics have recently been found in human blood and living lung tissue for the first time, the consumption of bivalves contaminated with microplastics could therefore be a direct entry route of these pollutants to humans.

The high concentrations of microplastics found at both sites (1274 microplastics kg^{-1} and 1125 microplastics kg^{-1}) which is more than double the estimated safe limits of sediment microplastic levels of 540 microplastics kg^{-1} (Everaert *et al*. 2018) may be linked to the shallow nature of this site accumulating these pollutants and Dundalk Bay serving as a hotspot for microplastic pollution. The assessment of microplastic pollution falls under descriptor 10 of the Marine Strategy Framework Directive which states that anthropogenic litter should not cause harm to the coastal or marine environment and that quantities of litter entering these environments should be reducing overtime (MSFD; 2008/56/EC). Currently there is no monitoring of quantities of micro litter entering the marine environment in Ireland. As the first country in the EU (following the UKs departure) to ban microbeads under the *Microbeads (Prohibition) Act 2019* which came into effect on the 20th of February 2020 Ireland was at the forefront of such actions against microplastics. While this is a positive step in limiting microplastic pollution, microbeads make up only a very small proportion of microplastics found in Irish environments and natural environments in general. The results of this study and others highlighting high proportional microfibre levels in Irish marine environments and biota illustrate the need for policy and legislative action against this form of pollution to the marine environment.

Chapter 5: Assessing microplastic pollution in marine communities of a shallow bay environment

Stephen Kneel*, Caroline Gilleran Stephens[1], Ana Marques Mendes[2], Liam Morrison[2] Suzanne Linnane[1]

[1]Centre for Freshwater and Environmental Studies, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland

[2]Earth and Ocean Sciences, School of Natural Sciences and Ryan Institute, National University of Ireland Galway, Ireland

*Corresponding author: stephen.kneel@dkit.ie

5.1 Abstract

The presence of microplastics is increasingly noted in marine species raising concerns for their potential to disrupt ecosystems and affect marine functioning. In this study microplastic presence is assessed in two different marine communities (intertidal and subtidal) found within the Special Protected Area (SPA) and Special Area of Conservation (SAC) of Dundalk Bay on the east coast of Ireland. Microplastics were extracted from specimens using 10% potassium hydroxide (KOH) and a subsample was analysed using Raman spectroscopy, confirming their anthropogenic origin. Microfibres were the most commonly found microplastic shape in 8 out of 9 species assessed and polyamide (PA) was the most common polymer noted. There was no significant difference in microplastics ind^{-1} between environs but microplastics g^{-1} were significantly greater in smaller intertidal species than subtidal ones indicating greater potential for negative effects from microplastic ingestion. The differences in concentration levels between species underline the importance of using an ecosystem-based approach in order to avoid under- or overestimating the microplastic contamination in biota for a study area.

5.2 Introduction

Microplastics or pieces of plastics regularly defined <5mm in size have become pollutants of environmental concern (Frias and Nash, 2019). Their small size makes them especially problematic given their bioavailability to a wide range of organisms and also the difficulty with their removal from the environment when compared to macroplastics (Dunham-Cheatham and Arienzo, 2022). Primary microplastics, those created to be of this small size, and secondary microplastics, those formed via breakdown of larger plastic material, are increasingly found in the environment with secondary microplastics the more prevalent type found (Hale *et al*. 2020). Recent studies have shown microplastics to be ubiquitous in the marine environment with their presence noted in; many species of marine animals (Lusher *et al*. 2018), near-shore environs (Harris, 2020 and references within), polar regions (Peeken *et al*. 2018; Isobe *et al*. 2017) and deep-sea sediment (Courtene-Jones *et al*. 2020). Microplastics can be ingested by a wide variety of species exhibiting different feeding mechanisms in the marine environment and can be consumed both accidently, such as via filtration feeding of bivalves, (Rochman *et al*. 2015) and through misidentifying them as prey items (De Sá *et al*. 2015). Microplastic contamination of field-collected benthic organisms including molluscs, crustaceans and polychaetes is noted (Martinelli *et al*. 2021; Vecchi *et al*. 2021; Sfriso *et al*. 2020).

When ingested and retained in gastrointestinal tracts of marine organisms microplastics have the capacity to damage or clog their tracts which can cause malnutrition via reducing food intake or causing false satiation (Walkinshaw *et al*. 2020). Capable of injuring organisms that consume them and accumulating environmental pollutants in addition to harbouring pathogens, (Cholewińska *et al*. 2022) microplastics can cause ecosystem-level effects, including the alteration of biogeochemical cycles (Seeley *et al*. 2020; Galloway *et al*. 2017).

While the term 'microplastic' was first coined by Thompson (2004) evidence of marine biota interacting with microplastics and associated concerns had been documented previously. Carpenter *et al*. (1972) noted 8 species of fish consuming polystyrene spherules 0.1 – 2mm in diameter in coastal waters of New England which raised concerns of intestinal blockage and physical injury in smaller fish. Microplastic pollution to intertidal and shallow subtidal species is not to be overlooked as these species play a key role in many marine tropic food webs as primary and secondary consumers in the exchange of nutrients and energy between the benthic food web and the pelagic food web (Sinha *et al*. 2021). The threat of microplastics to benthic species is particularly concerning since they contribute up to 90% of fish prey biomass (Hägerbäumer *et al*. 2019; Schindler and Scheuerell, 2002; Weber and Traunspurger, 2015) leading to the possibility of larger fishes being exposed to microplastics through contaminated prey raising concerns for bioaccumulation and biomagnification in marine biota (Barboza *et al*. 2018), with microplastics noted in the muscle tissue of large predatory fish that are consumed by humans (Di Giacinto *et al*. 2023). Many species of edible demersal, pelagic and reef fish, sampled from across the globe, have been found to ingest microplastics (Walkinshaw *et al*. 2020 and references within). Recently microplastics have been documented in benthic species from various marine ecosystems exhibiting different feeding patterns (Sfriso *et al*. 2020; Coppock *et al*. 2021; Joyce *et al*. 2022). Furthermore, ingestion of microplastics by marine filter feeders can have more severe effects on food chains as they are their baseline (Khalid *et al*. 2021). The ingestion of microplastics can lead to adverse effects such as oxidative stress, cell damage, tissue inflammation, increased gut residence times and the leaching of chemical additives and sorbed contaminants (Vethaak and Leslie, 2016; Gray and Weinstein, 2017).

Microplastics have become the subject of increased environmental studies both examining their presence in the natural environment and wild-caught biota and through eco-toxicological lab work (e.g., Nunes *et al*. 2023; Tran *et al*. 2023). While much work has been carried out on species that have economic value to humans (Walkinshaw *et al*. 2020) less work, however, has been conducted on species that have limited or no-commercial value, including species of invertebrates. This presents an oversight in data acquisition as invertebrates serve as important prey species for commercially valuable species as well as existing as primary consumers in food webs and may represent starting points for microplastics entering marine food webs. An example of such gaps in knowledge is the lack of microplastic contamination research conducted on the deposit feeder, *Echinocardium cordatum* despite the fact it is among the most studied irregular echinoid for biology, ecology and morphology in general, and is likely the most widely distributed extant echinoid (Mortensen, 1951; Hyman, 1955; Higgins, 1974). Furthermore, little research exists documenting microplastic presence in predatory gastropods despite the fact their typical prey species, bivalves are one of the most widely studied group of marine species with respect to microplastic presence (Ding *et al*. 2022). Furthermore, it has been suggested that predators and deposit feeders may be more susceptible to ingesting plastic particles on seabed sediments (Bour *et al*. 2018; Naji *et al*. 2018) as marine sediment is a sink for microplastics (Mason *et al*. 2022). A recent review of available literature by Walkinshaw *et al*. (2020) indicated that microplastics are more prevalent in lower trophic organisms.

Coastal ecosystems serve as important nursey zones for juveniles of many fish species (Cheminée *et al*. 2021). During their lifetime the juvenile habitat is often only a small portion of the habitat that individuals of a fish species will occupy (Gibson, 1994, Elliott and Dewailly, 1995). However, the threat posed by microplastic pollution and exposure may be heightened in the juvenile stage of a fish's life cycle. Smaller sized fishes are likely more prone to misidentify microplastics as prey items (Ory *et al*. 2017) in addition, they are more likely to experience mechanical interference in their feeding and digestion processes than larger sized fishes (Jovanović, 2017). There is a high likelihood that juvenile fish living in estuarine environments will interact with microplastics as these habitats are usually located close to contamnination sources (Naidoo *et al*. 2015, 2016). Worringly, microplastic exposure resulted in the formation of intestinal lesions in the intertidal fish species *Girella laevifrons* (Ahrendt *et al*. 2020). Real-world microplastic contamination is documented in juveniles previously, for example; 100% of juvenile fishes of 3 silverside species recovered from tidal pools contained microplastics in their stomachs (Mendoza *et al*. 2022) and 70% of Patagonian blennies (*Eleginops maclovinus*) contained microplastics (Mendoza *et al*. 2023).

Estuaries and near-shore or intertidal habitats, being first to receive river inflow transporting contaminants into surface waters, are sites of potentially high microplastic contamination given their proximity to terrestrial environments where as much as 80% of marine litter originates (European Environment Agency, 2023; Browne *et al*. 2010; Wright *et al*. 2013). Due to their proximity to the terrestrial environment, these ecosystems are at a wider risk of anthropogenic pressures disturbing them. Additionally, recent modelling work indicates that approximately 77% of positively buoyant marine plastic litter stemming from land-based sources spends 5 years beached or floating in coastal water (Onink *et al*. 2021). Furthermore, degradation and fragmentation of plastic into microplastic form is expected to be greatest in surface water and on beaches where the rate of solar UV-induced photodegradation is greatest (Cooper and Corcoran, 2010). Furtermore, microplastic pollution has been noted in a large number of marine intertidal locations (Perfetti-Bolaño *et al*. 2022; Mendes *et al*. 2021; Bucol *et al*. 2020) and surveys have found the highest regional macro- and microplastic concentrations on the seafloor around estuarine inputs (Galgani *et al*. 2000; Browne *et al*. 2010).

Soft-bottomed benthic environments can act as both a sink and source of microplastic pollution (Brown *et al*. 2011). Microplastics can settle on the seabed as their density increases due to a variety of processes and end up settling on the seabed such as; biofilm formation (Lobelle and Cunliffe, 2011), egestion as part of a faecal pellet (Cole *et al*. 2013), or flocculation and sinking as aggregates (Long *et al*. 2015; Bergmann *et al*. 2017; Michels *et al*. 2018). However, wave-induced bottom currents in shallow nearshore regions have the ability to resuspend this material in the overlaying water body (Johnson *et al*. 1987; Muchane, 1994). As approximately only 10% of plastic marine litter remains at the sea surface (Andrady, 2015) and it appears that only a small amount of microplastics is reaching the deep-sea environment (Harris, 2020) it is likely that a large amount of microplastic litter settles in near-shore shallow environments.

Fauna in benthic environments can interact with microplastics in a number of ways. The sediment-dwelling brittlestar, *Amphiura filiformis*, has been shown to bury microfibres both by passively transporting downwards when maintaining their burrows but also through ingestion and egestion (Coppock *et al*. 2021). The cockle, *Cerastoderma glaucum*, emerged from sediment less often and in lower numbers in sediments containing large and medium-sized microplastics in higher concentrations while the Baltic clam, *Limecola balthica*, burrowed into deeper sediment layers in microcosms treated with microplastics than in control sediments (Urban-Malinga *et al*. 2021). Furthermore, a clear prey potential pathway of microplastics into benthic food webs has been shown where lobsters (*Nephrops norvegicus*) fed with fish that had been fed with fishing net-based fibres were shown to accumulate microfibres in their stomachs (Murray and Cowie, 2011).

Temperature estuarine and coastal systems serve as nursery areas for various fish and crustacean species and as feeding grounds to coastal and migratory birds (Berman *et al*. 1988; Piersma, 1994; Zijlstra, 1972). It is important therefore, to study the presence of microplastics in species found in shallow coastal environments of Dundalk Bay as it is a productive ecosystem serving as a nursery site for juveniles of large fish species before they move onto deeper waters and hosting a range of organisms some of which are of commercial value. Indeed, Dundalk Bay is a vital nursery area for all the commercial fish species in the Irish Sea and is anecdotally considered the most important nursery area along the east coast of Ireland (Linnane *et al*. 2022). This was highlighted in numerous fish surveys conducted showing that the fish species composition in Dundalk Bay shallow littoral zone and subtidal zone is primarily made up of migrant marine species (Connor *et al*. 2019). *Gobiiformes* (sand goby) and *P. platessa* were recorded amongst the ten most common species found in temperate estuaries in a nationwide study on Irish coastal waters (Connor *et al*. 2019).

Dundalk Bay is a unique habitat in that it is a very large and shallow bay hosting an extensive intertidal zone and also shallow subtidal environment just before the Bay opens into the Irish Sea (Dundalk Bay SAC (site code 455). The shallow nature of Dundalk Bay, affecting dilution factor, the proximity to the terrestrial environment and presence of fishing activity means that fauna found here are potentially at heightened risk to microplastics (Kye *et al*. 2023; Graca *et al*. 2017). Additionally, as chapter 3 of this thesis displayed microplastics are transported in freshwater waters that flow into this marine environment. The marine community of the bay is highly interlinked with many species serving as food sources to the large populations of overwintering waterbird that reside here, additionally, overlapping prey spectra for many species could result in similar, heightened microplastic exposure levels in the inshore region. After a (re-)colonisation period by post larval juveniles or by adult stages in summer, shallow-water coastal bodies support abundant epibenthic assemblages exploiting suitable food sources (Freitas *et al*. 2011). The intense use of these areas by various epibenthic species combined with the similarities in prey spectra (Evan, 1983; Pihl, 1985) mean that microplastic contamination levels may be similar between species as indiscriminate feeding occurs.

While marine species have been assessed for microplastic contamination in Irish waters previously (e.g., Joyce *et al*. 2022; Pagter *et al*. 2021) less work has been done on species inhabiting shallow environments, highlighting a knowledge gap given that the likelihood of interaction with microplastic in these environs is greater. Furthermore, the majority of microplastic studies examining contamination in biota target one species which has the potential of under- or overestimating microplastic levels in the environment (Valente *et al*. 2022; Pagter *et al*. 2020b) while also being focused on species of commercial value whereas a mix of commercially important and not commercially important species are examined as part of this study.

The aims of this study therefore were to assess microplastic concentrations in several species found in the shallow marine environment of Dundalk Bay and examine the relationship to the habitat (intertidal vs subtidal) and feeding mechanism. The present study investigates microplastic occurrence in the species of two different habitats found within a bay environment; an intertidal site and a shallow subtidal zone and examines both commercial species and non-commercial species. Microplastic presence is assessed per individual (MPs individual^{-1}) and per gram wet weight (MPs g^{-1}) and trends related to the weight and length of fishes are examined.

5.3 Material and methods

5.3.1 Study area

The focus of this study were species found in Dundalk Bay (Latitude: 53.9586 Longitude: -6.33845). Dundalk Bay is a semi-enclosed bay located on the East coast of Ireland. Dundalk Bay is bordered by the mountainous region of Cooley peninsula to the North and the area of Annagassan to the South. While the hydraulics of the Bay are dominated by the sea, the rivers; Fane, Big, Castletown, Glyde and Dee are major sources of freshwater to the Bay environment. The Bay fills with water from the Irish Sea from the South with the tide approaching in a clockwise manner. The inner-bay is shallow, sandy and intertidal with up to 70% of its surface exposed during low tide. The Bay is a classified shellfish production area for both razor clams (*Ensis siliqua*) which are collected from deeper waters and cockles (*Cerastoderma edule*) harvested from the shallower areas (BIM 2022, shellfish stocks and fisheries review). There are several potential sources of microplastics to this environment. These sources include; the two urban wastewater treatment plants located on the bay, one on the coast of the suburb Blackrock (Population equivalence (PE) 7,300) and one at Soldiers Point where the Castletown River enters Dundalk Bay (PE 61,000). In addition, potential microplastic sources of concern to the Bay include; agricultural land where biosolids have been applied (treated sewage sludge) which may run-off to river bodies or the bay itself, the urban centres of Dundalk and the growing suburb of Blackrock to its South and various commercial fishing activities that occur in the Bay. Dundalk Bay qualifies as a Special Protected Area (SPA) (site code: 004026) and Special Area of Conservation (SAC) (site code: 000455) possessing a rich diversity of habitats including: marine waters, saltmarshes, estuaries and extensive sand and mud flats (extending over 4000 ha) which have a rich fauna of molluscs, polychaetes and crustaceans that provide an important food source for the bay's wintering waterfowl (Dundalk Bay SPA (004026): Conservation objectives supporting document). Dundalk Bay is a Natura 2000 site and protects 23 species under the Nature Directive and 6 habitat types under the Habitats Directive (EUNIS -Site factsheet for Dundalk Bay SAC). Currently, however, there are no Water Framework Directive High Status Rivers flowing into the Bay, with Louth one of only two counties in the ROI that have no rivers of ecologically high status (EPA, 2021). Additionally, as shown in chapter 3 microplastics in the surface waters of the rivers that enter Dundalk Bay have been documented thus depositing terrestrial microplastics directly into this marine environment. Furthermore, the contamination of the commercially and ecologically valuable bivalve, *Cerastoderma edule*, collected from Dundalk Bay with microplastics, which is a prey item of several of the species examined in this work has been shown in chapter 4. The juveniles of the commercially important species, *P. platessa* shelter in this environment before moving further offshore upon reaching maturity. Approximately 574 t of plaice were caught and landed annually in mixed demersal fisheries by Irish vessels between 2016 and 2018 and the species is mainly caught as bycatch in coastal, shallow, sandy areas (Marine Institute, 2019; Oliver *et al*. 2020). The bivalve, *E. siliqua* is commercially important and landings of this species were stable between 2020-2022 at approximately 500 tonnes per year with Dundalk Bay one of two production areas that amount for the majority of total landings nationwide (BIM 2022, Shellfish Stocks and Fisheries Review).

5.3.2 Study organisms – Intertidal

5.3.2.1 *Pleuronectes platessa*

Newly metamorphosed plaice, *Pleuronectes platessa*, are abundant in shallow inshore waters from the shoreline to a depth of 10m and feed on small polychaete worms, harpacticoid copepods, amphipods, crab larvae and small molluscs (Rijnsdorp and Pastoors, 1995; Mariani *et al*. 2011) and it is not uncommon to find juveniles of this species in sandy intertidal shore-pools (Wheeler, 1978; Frimodt, 1995). Competition with *gobiidae* and epibenthic crustaceans has been attributed as the most likely underlying mechanism responsible for observed growth reduction in 0-group *P. platessa*.

5.3.2.2 *Crangon crangon*

Juvenile post-settlement brown shrimp (*Crangon crangon*) migrate to inshore nursery areas for better foraging and protection from predators and typically remain here for several weeks before returning to deeper offshore waters (Cattrijsse *et al*. 1997). This species will feed on most animal material including polychaetes, fish, molluscs and small arthropods and also algae (Dolmer *et al*. 2001; Henderson & Holmes, 1987; Kamermans & Huitema, 1994; Oh *et al*. 1999). Given its voracious appetite and indiscriminate feeding mechanism the selection of prey is noted to be size-dependent and related to relative abundance of prey and cannibalism has also been noted in this species (Pihl and Rosenberg, 1984). The major predators of both juveniles and adults of this species are juvenile fish such as whiting (*Merlangius marlangus*) and cod (*Gadus morhua*) (Kühl, 1964; Tiews, 1965), however, smaller fish species such as the sand goby (*pomatoschistus minutus*) feed on small juveniles Hamerlynck & Cattrijsse, 1994; Salgado, Nogueira Cabral, & Costa, 2004). Furthermore, swimming crabs of the genus *Liocarcinus* are noted predators of recently settled juveniles that have just switched from the pelagic larval to the benthic phase with up to 50% of the foregut content of *Liocarcinus holsatus* consisting of juvenile *C. crangon* (Choy, 1986).

5.3.2.3 Gobiidae

Gobies, *gobiidae*, are one of the most frequent group of bottom-feeding fish in the Irish Sea and should be regarded as important predators of shallow marine environments. Although their small size means they are not of commercial value in terms of human diet (Berge and Hesthagen, 1981), gobies serve as intermediate predators in marine food webs and serve as important prey for larger demersal fish and predators of small benthic and epibenthic organisms (Freitas *et al*. 2011). Sand gobies, *P. minutus*, are generalist and opportunistic predators and take prey items based on their availability (Evans and Tallmark, 1985; Pihl, 1985; Salgado *et al*. 2004). Gobies exhibit a feeding mechanism known as “sit-and-wait” laying down at the bottom of the substrate until a prey comes along (Magnhagen, 1986) and filial cannibalism (i.e., the predation on one’s own offspring) is exhibited by male gobies (Chin-Baarstad *et al*. 2009; Kvarnemo *et al*. 1998). The feeding ecology of the sand goby *P. minutus* and the common goby *P. microps* has been assessed in the upper Tagus estuary in Portugal. It was noted that *P. microps* preferentially ingested polychaetes, with isopods, amphipods, bivalves and copepods taken as secondary prey items while the sand goby had no dominant prey, although mysids were particularly important in the diet of this species (Salgado *et al*. 2004).

5.3.2.4 *Lanice conchilega*

The sand mason worm, *Lanice conchilega*, is a polychaete worm that reaches lengths up to 30cm and makes a tube of sand grains and shell fragments with a frayed end which sticks up above the sand (Vlaminck *et al*. 2023) and feeds via deposit feeding or suspension feeding displaying behavioural plasticity when the population density is high (Buhr end winter, 1977; Buhr, 1976; Holtmann *et al*. 1996).

5.3.3 Study organisms – Subtidal

5.3.3.1 *Ensis siliqua*

The razor clam, *Ensis siliqua*, is an abundant marine bivalve widely distributed on the East Coast of Ireland (Fahy, 1999). It can be found in depths up to 58m, however, they are thought to be most common in shallower waters 3 – 7m in depth (Costa *et al*. 2010; Encyclopaedia of Life, 2010; Fahy, 1999; Gaspar and Monteiro, 1998). The species is of commercial value and is harvested via dredging by commercial fisheries in Spain, Portugal and Ireland (Costa *et al*. 2010), in Ireland, annual first sale value is approximately €6m (Marine Institute, 2016). Native *Ensis* spp. are longer lived species generally in excess of 10 years (Woolmer, 2007) and individuals of up to 19 years recorded in Ireland (Fahy and Gaffney, 2001). They bury themselves vertically in the substrate using their large and powerful foot and use their siphons for suspension feeding on particulate organic matter, principally, phytoplankton (Breen *et al*. 2011). Large densities of *Ensis* sp. are believed to reduce growth rate and hinder settlement as young razor clams struggle to compete for food and space and adults can be predators of their own larvae (Hauton *et al*. 2011). Diving seabirds such as scoter, *Melanitta nigra* and eider duck, *Somateria mollissima* prey on razor clams (Aitken and Knott, 2018). Exposed clams are also preyed upon by several species of crab, the harbour crab, *Liocarcinus depurator* among them (Fraser *et al*. 2018). Additionally, the sand goby, *P. minutus*, are known to attack exposed razor clams (Robinson and Richardson, 1998; Murray *et al*. 2014). The edible crab, *Cancer pagurus*, is thought to be the main predator on this species in many areas (Tuck *et al*. 2010) as it is able to excavate them from the substrate in which they are buried (Hall *et al*. 1991) while the starfish, *Marthasterias glacialis*, has been observed extracting razor clams from their burrows (Breen *et al*. 2011). However, individuals exposed via dredging may be susceptible for predation by a wider range of species than would naturally target them such as scavenging fauna or opportunistic predators such as crabs (Aitken and Knott, 2018) and their potential predator pressures are myriad.

5.3.3.2 Liocarcinus depurator

Portunid crabs or swimming crabs include over 300 mainly predatory species and the diet of temperate species varies in relation to the lower diversity and seasonal availability of prey in these regions unlike in tropical or sub-tropical regions (Choy, 1986; Careddu *et al*. 2017). The harbour crab *Liocarcinus depurator* occurs in the waters of the Mediterranean and the Northeast Atlantic shelf and is one of the most common portunid crabs found in these waters (Mori and Zunino, 1987; Abelló *et al*. 1988; Rufino *et al*. 2004) and due to this abundance can play a key-role in soft-bottom community structuring via species suppression and being a prey species for fish and other predators (Careddu *et al*. 2017). Harbour crab feeding ecology has been assessed in the past. Freire (1996) found crustaceans, molluscs, polychaetes, ophiuroids and fishes constituted most of the diet of crabs sampled from the inner and outer channel stations while predation on several groups of molluscs varied greatly among habitats assessed in Riá de Arousa, Spain. Harbour crabs sampled two different subtidal areas of the Gulf of Gaeta, Italy found that polychaete worms, amphipods and bivalves were consumed by crabs at both locations but in differing compositions (Careddu *et al*. 2017).

5.3.3.3 Polinices catenus

Naticid gastropods are a family of predatory small-medium sized marine snails commonly known as moon shells or necklace shells, are distributed worldwide (Kabat, 1990). They are shallow infaunal living snails and have been a major source of mollusc mortality since the Cretaceous era (Sohl, 1969). Prey are enveloped by the mesopodium and orientated to a preferential position for drilling by the radula with secretions of the accessory boring organ which depending on shell thickness can take hours to days to complete, following drilling completion ingestion occurs via the proboscis inserted through the drill hole (Carriker, 1981; Kelley and Hansen, 1993). While naticid gastropods attack both infaunal and epibenthic prey, they always drill their prey within the sediment (Mondal and Harries, 2013; Sohl, 1969; Carriker, 1981). While generally actively preying in shallow to deep water there are instances of naticid gastropods invading exposed intertidal areas and preying upon molluscs have also been reported (Gonor, 1965; Hughes, 1985; Savazzi and Reyment, 1989). Common cockles, *Cerastoderma edule* are common prey species of gastropods of this order, however, many types of bivalves are also consumed (Ansell 1982; Kinglsey-Smith *et al*. 2003) and cannibalism within naticid gastropods also occurs (Kelley and Hansen, 1993). For example, juveniles of *Polinices catena* feed on bivalves, other gastropods and by cannibalism (Ansell, 1982). Naticid gastropods select larger prey as they themselves increase in size (Edwards and Huebner, 1997; Griffiths, 1981; Berry, 1982; Rodrigues *et al*. 1987) and can shape the communities of soft-bottomed marine environments by regulating the abundance of prey molluscs (e.g., Aristov and Varfolomeeva, 2019).

5.3.3.4 *Echinocardium cordatum*

Echinocardium cordatum, a heart sea urchin, known colloquially as sea potatoes are common in coastal waters of both Southern and Northern hemispheres and is likely the most widely distributed extant echinoid (Mortensen, 1951; Hyman, 1955; Higgins, 1974), furthermore, it is among the most widely studied irregular echinoid in terms of ecology, biology and morphology (Ridder and Saucéde, 2020 references within). *E. cordatum* can be found from the intertidal zone down to the subtidal and depths of approximately 250m with most populations well offshore (Ursin, 1960) and lives buried in marine sediment usually from 4 to 20cm in depth (Ridder and Saucéde, 2020 references within). The urchin lives in a burrow that is connected to the surface of the substrate by a vertical funnel which is wider at the top. *E. cordatum* is an infaunal deposit feeder, ingesting sediment in bulk from the sea floor and feeding on the particulate organic matter occurring between the sediment grains (Brafield, 1978; Morton, 1979). The biomass of the species varies with habitat type (Duineveld and

Jenness, 1984) but it may account for up to 60% of the total benthic biomass (e.g., Rees, 1954; Duineveld and Jenness, 1984; Nakamura, 2001) with the species being an important contributor to the macrofaunal community (Dauwe *et al*. 1989). There are several pronounced predators of *E. cordatum*, namely; by asteroids (*Astropecten*) (Sloan, 1980) and demersal fish like plaice (Carter *et al*. 1991) in offshore locations and by gulls on shallowly buried individuals (Ridder and Saucéde, 2020). The species is relatively long-lived at 10-20 years (Ursin, 1960; Buchanan, 1966; Duineveld and Jenness, 1984). It is used for marine bioassays for assessing reburial activity an survival due to chemical contamination of marine sediments (Bowmer, 1993; Daan and Mulder, 1996; Stronkhorst *et al*. 1999; Brils *et al*. 2002). In coastal European waters it is routinely used for the assessment of sediment quality and screening contaminated dredged material that is proposed for open water disposal (Stronkhorst *et al*. 2003).

5.3.3.5 Aphrodita aculeata

The marine annelid worm known as the sea mouse, *Aphrodita aculeata* is distinguished by the conspicuous layer of long, fine chaetae forming a mat of felt that cover the scales of the species. Currently there is limited information on this species available with the observations by Mettam (1980) the most important. The species feeds on worms both sedentary species and sessile ones, very young crabs and small hermit crabs (Mettam, 1980). Observations of the species by Mettam (1980) found that prey were only taken when they (*A. aculeata*) were buried and was capable of consuming much larger prey than themselves with the feeding on the king rag, *Nereis virens*, likened to “a hedgehog swallowing a snake” (Gunnar Thorson pers comm. cited in Mettam, 1980). *A. aculeata* is a known dietary component of the cloudy catshark, *Scyliorhinus torazame* (Park *et al*. 2019) and sharks of the Western Mediterranean Sea (Barría *et al*. 2018).

5.3.4 Field sampling and species selection

Subtidal species specimens were collected by local fishermen as bycatch from the harvesting of cockles from Dundalk Bay via dredging (Fig. 5-1, Table. 5-1). Benthic invertebrates were collected immediately after the fishermen returned to shore, transported to the laboratory, sorted to species level, wrapped in aluminium foil and stored in metal food containers before being frozen at -20°C for future analysis. The 5 species examined for microplastic contamination were; the polychaete, *Aphrodita aculeate* (sea mouse), the bivalve, *Ensis siliqua* (razor clam), the crab, *Liocarcinus depurator* (blue-leg swimming crab) urchin *Echinocardium cordatum* (sea potato) and the gastropod, *polinices catenus* (necklace shell). Species recovered from the intertidal area in the South of Dundalk Bay were collected via Seine-netting on a retreating tide, beginning at a depth of approximately 50cm. Specimens were taken from the results of 3 netting procedures. The 4 species assessed for microplastic pollution in the intertidal inner shore area were; *Crangon crangon,* (brown shrimp), *Pleuronectes platessa* (plaice), *gobiiformes* sp. and the protruding section of the tube of the sand mason worm, *Lanice conchilega.* Beach Seine netting was conducted using a 30m x 3m net (10mm mesh size) to capture fish in littoral areas. The bottom of the net has a weighted lead line to increase sediment disturbance and catch efficiency.

These species were selected as they represent different feeding modes and occurred in different marine environments in Dundalk Bay potenitially exhibiting different exposure levels to microplastics in order to give a balanced insight of microplastic contamination levels for this environment. The feeding modes represented include; filter-feeding (*E. siliqua*), surface-deposit feeding (*E. cordatum*), opportunistic (*L. depurator*, *A. aculeata*) (Hill, 2008; Mettam, 1980) and active predation both within the sediment (*P. catenus*) and above it (*P. platessa, gobiiformes, C. crangon*). Additionally, the tubes of the sand mason worm (*L. conchilega*) were examined for microplastic presence in order to see if they are incorporated into their construction as grains of sand and pieces of shell are.

These species were selected as they represent a diverse range of feeding strategies which will help to gain an insight into what species are more prone to ingest microplastics in shallow marine environments in close proximity to microplastic sources. In this study juveniles of the commercially important species *P. platessa* were examined for microplastic pollution as past studies have documented their presence in digestive tracts of adults collected in deeper waters (Welden *et al*. 2018), however, there is a lack of knowledge on the presence in juveniles which is where the effects of microplastic ingestion may be most severe. Two other species, *Gobiiformes* sp. and *C. crangon* were also studied as they have overlapping diets with juveniles of *P. platessa* occurring in the same marine habitats in order to establish if microplastic consumption is heightened in juveniles of *P. platessa* when compared to species with similar diets. Previous work has suggested that deposit feeders may more likely to ingest microplastics given their feeding at the seafloor (Naji *et al*. 2018), however, this has not been documented in *E. cordatum* despite their importance in bioturbating marine sediment and likely being the most widely distributed extant echinoid (Riddler and Saucéde, 2020). The razor clam, *E. siliqua*, was studied for microplastic pollution in this study as in chapter 4 of this thesis another filter-feeding bivalve, the common cockle, *C. edule* has been documented to contain microplastics, however, it is typically found in shallower waters and so *E. siliqua* may have potential as a biomonitor for microplastic presence in deeper marine environments. Predatory marine gastropods are an understudied group of species in terms of microplastics research despite the fact their prey species, bivalves are possibly the most widely studied group of species (Ding *et al*. 2022) which represents a knowledge gap. Recently it has been shown that microfibres can transfer to the carnivorous gastropod *Reishia clavigera* through feeding on mussels (Xu *et al*. 2022) thus highlighting potential routes of microplastic trophic transfer in marine food webs. The sea mouse, *A. aculeata* is a relatively understudied polychaete, feeding below the sediment (Mettam *et al*. 1980) with an opportunistic diet consisting of other polychaetes and small crustaceans but will also feed on carrion, which can result in a diverse range of microplastic exposure pathways via its feeding mechanisms. Freshwater invertebrates have been documented incorporating microplastics into their casings in several studies (e.g., Alvarez Troncoso *et al*. 2022; Ehler *et al*. 2020; Tibbets *et al*. 2018) while limited studies are available on the presence of microplastics in tubes of marine worms (Piazzolla *et al*. 2020; Knutsen *et al*. 2020) in order to add to this body of work the tubes of the sand mason worm *L. conchilega* were assessed in this study. An overview of species studied and collection method for each habitat is displayed in table 5-1.

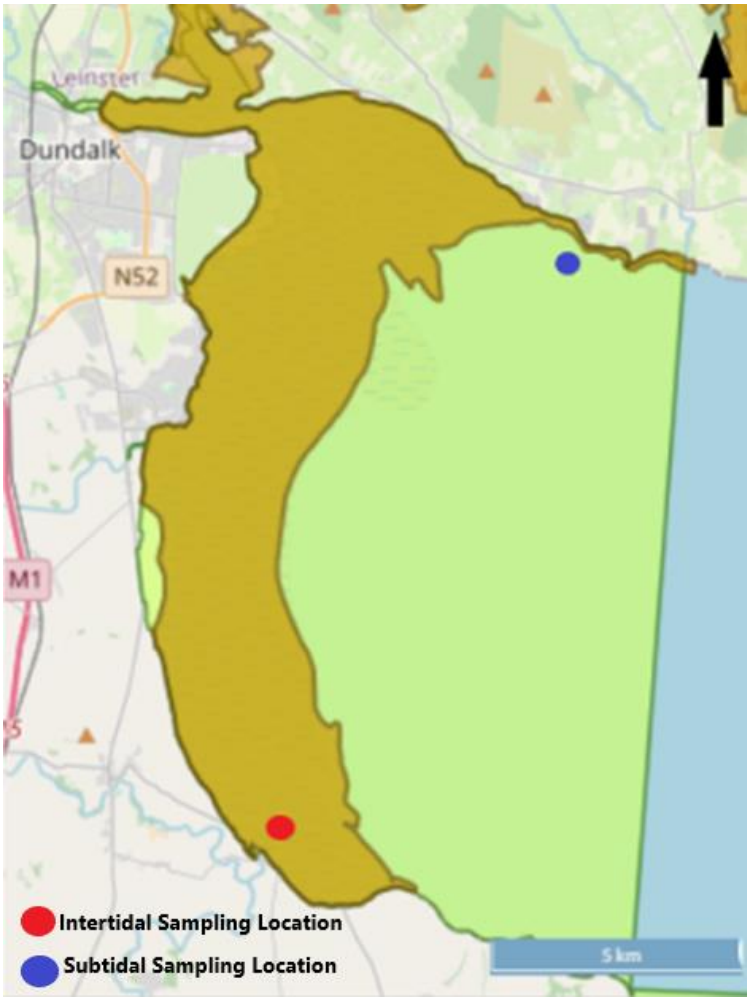


Figure 5-1: The SPA of Dundalk Bay highlighted in Green overlapping the area of the SAC in brown. The area where bycatch was collected from indicated with a blue circle and Seine-netting for intertidal species was carried out at the location marked with a red circle.

Table 5-1: Species and their associated marine habitats in Dundalk Bay.

Environment	Substrate Type	Sampling method	Species recovered
Subtidal	Soft-bottom sand	Bottom dredging	*Polinices catenus, Ensis Siliqua, Echinocardium cordatum, Aphrodita aculeate, Liocarcinus depurator*
Intertidal	Soft-bottom sand	Seine Netting	*Crangon crangon, Lanice conchilega, Pleuronectes platessa, Gobiiformes* sp.

5.3.5 Dissection, digestion and examination of marine organisms

Following defrosting the biometrics of all species were recorded (weight in shell, length, width). Dissection of species was carried out as per Fang *et al*. (2018). For *L. depurator*, the carapace was cut open using surgical scissors and soft tissue was removed including gills and visceral mass. *E. siliqua* samples were opened and all soft tissue was removed for digestion. *E. cordatum* shells were opened and all internal material was removed. *C. crangon* were combined in groups of 5 in order to form composite samples and underwent homogenisation using a mortar and pestle. *P. catenus* were removed from their shells using tweezers. *A. aculeata, gobifformes* sp., *P. platessa* and *L. conchilega* tubes underwent no dissection.

Prior to digestion, the wet weight of material was recorded, rinsed with Milli-Q water (0.22µm) and placed in Erlenmeyer flasks. Digestion protocol was followed as per Thiele *et al*. (2019). Briefly, KOH 10% was used to digest all study organisms. Flasks were incubated at 40ºC at 250 rpm in an oscillation chamber for 24 hours. Prior to filtration solutions were neutralised using 1M citric acid. For the species *E. siliqua* and *E. cordatum* an extra extraction step was necessary following digestion given the large amount of sediment present in these species. For these two species following digestion, the supernatant was decanted for filtration slowly. When as little digested liquid as possible was remaining, samples were placed in an oven at 40ºC until this evaporated. Following this potassium carbonate (K_2CO_3) with a density of 1.54 g/cm^3 (Gohla *et al*. 2021) was added to the remaining material, mixed with a magnetic stirrer for 2 minutes and left to settle for 15 minutes before the supernatant was filtered (Whatman, 47mm diameter GF/C 1.2µm glass microfiber). Filter papers were placed in labelled petri dishes after filtration and stored in a desiccator prior to visual examination.

Once filter papers were adequately dried, they were examined under a microscope (Olympus SZX7) and microplastics were counted. As per Gewert *et al*. (2017) metal tweezers were used to test the consistency of suspected microplastics. Plastics are generally firmer than organic material such as leaves or algae and inorganic material such as a sands crumble when pressure is applied and characterised based on their response to metal tweezers (plastics should not break under stress but should flex or bend) (Keene and Turner, 2023) and microplastics were measured. In terms of shape, microplastics were classified as one of; fibre, films, fragments or bead. Colours of microplastics were also noted and red, blue, green, transparent / white, multi-coloured and black were recorded while other less common colours were classed as “other”. In order to allow comparisons between shapes and colours of microplastics microscope work was carried out by an individual operator for this study.

5.3.6 Quality control measures

All handling and processing of samples was completed by a lone operator in a designated clean room solely used for microplastic work in which only one individual was allowed to work at any time. A 100% cotton lab coat was worn when conducting all lab-work. In general, processing was carried out under a laminar flow hood in which clean filter papers were placed in open petri dishes in order to account for any potential airborne contamination. A sticky-mat was placed outside of the entrance into the clean room (Multi-Layer Sticky / Tacky EnviroTack™ Mats - 18x36 inch) which helped to mitigate contaminants entering on the shoes of the operator. The clean room was vacuumed before the beginning of any lab work and following this work surfaces were wiped down using 70% ethanol solution and Milli-Q water (0.22µm) with cotton wipes made from long stable cotton yarn to eliminate free-floating fibres on fabric surfaces (Cleanroom wipes, Texwipe®). All solutions used in the study were pre-filtered (Whatman, 47mm diameter GF/C 1.2µm glass microfiber) before use with samples. Procedural blanks were included per set of samples processed in order to quantify background contamination. All glassware and steel equipment was triple rinsed with Milli-Q water (0.22µm) and covered in aluminium foil when not in use. Results from blanks and air controls were compared to concentrations found in study organisms through paired t-tests and found to be statistically different for all organisms as prescribed by Dawson *et al*. (2023) and no correction methods have been applied to them. Potential laboratory-introduced contamination of each species is displayed in Appendix C: Table 8-8.

5.3.7 Statistical analysis

Microplastic abundances data (MPs ind^{-1} and MPs g^{-1}) was assessed for normality using the Anderson Darling test. The results of both sets of data displayed that they were not normally distributed. Both data sets were transformed using the Johnson transformation. A one-way ANOVA (analysis of variance) was performed to test how these factors varied between species and how they differed based on feeding mechanisms and where differences occurred Tukey's post-hoc testing was conducted. Independent t-tests were performed to assess if microplastics abundances (MPs ind^{-1}

and MPs g^{-1}) were significantly differently between habitats assessed (intertidal vs subtidal). Statistical significance was accepted at $\alpha = 0.05$ for all tests.

Linear and polynomial regression analysis was carried out to examine the relationship between length and microplastic burden for the species; *E. siliqua*, *P. platessa*, *A. aculeata* and *gobiiformes* sp. and also wet weight and microplastic burden for all species examined in this study. Data was analysed statistically using the Minitab statistical software package (version Minitab® 21.1.1 (64-bit)) and Microsoft Excel 2016. Minitab statistical software package was also used to create graphs of results.

5.3.8 Raman analysis

A subsample of microplastics from digested specimens was selected for characterisation and polymer identification using Raman Spectroscopy. The Raman Spectrometer was equipped with a 600 groove mm^{-1} diffraction grating, a confocal optical system, a Peltier-cooled CCD detector, and an Olympus BX41 microscope (Ó Briain *et al*. 2020; Loughlin *et al*. 2021) and spectra were obtained at a range of 100–3500 cm^{-1} using a 532nm laser. Spectra obtained when analysing particles extracted from marine species were compared to a spectral reference library (KnowItAll, Bio-Rad), an in-house extension of the library with additional spectra from environmental plastics collected from the intertidal zone and known virgin polymer types (purchased from CARAT GmbH, Bocholt, Germany) (Mendes *et al*. 2021). In addition, SLoPP and SLoPP-E libraries (Munno *et al*. 2020) were employed, and the 'fingerprint' region of each spectrum was used to identify the polymer type. The website 'Open Specy' (Cowger *et al*. 2021, (https://openanalysis.org/openspecy/) in addition to 'PublicSpectra' (https://publicspectra.com/SpectralSearch) was also used to verify polymer type. Furthermore, the Infrared & Raman Users Group (http://www.irug.org/search-spectral-database) was also consulted.

5.4 Results

5.4.1 Microplastics in subtidal and intertidal species

A total of 870 microplastics were extracted from 120 individuals and 10 composite samples across the 9 different species examined. 330 microplastics were recovered from species in the intertidal zone (n = 54) while 540 microplastics were recovered from species inhabiting the shallow subtidal habitat (n = 76). A variety of colours, shapes and polymers were identified.

In terms of MPs g^{-1}, species found in the subtidal environment had lower values (average; 1.36 ± 1.94 MPs g^{-1}) than the intertidal (average; 12.02 ± 8.34 MPs g^{-1}). From species recovered from the subtidal environment, the crab, *L. depurator* had the highest concentration of microplastics (4.28 ± 4.0 MPs g^{-1}) while the bivalve, *E. siliqua* had the lowest (0.31 ± 0.19 MPs g^{-1}). *P. platessa* (14.8 ± 5.58 MPs g^{-1}) had the highest microplastic contamination of species found in the intertidal zone while *C. crangon* (5.81 ± 2.83 MPs g^{-1}) had the lowest. One-way ANOVA testing with Tukeys post-hoc analysis revealed the mean microplastic concentrations of *P. platessa* were statistically significantly greater than all other species that had ingested microplastics except *Gobiiformes* sp. and was similar to the concentrations found in the casings of *L. conchilega*. Concentrations of microplastics in *E. siliqua* were significantly lower than every other species except for *A. aculeata* (Fig. 5-2). Additionally, two outliers were noted, one amongst *L. depurator* (14.33 MPs g^{-1}) specimens studied and one amongst *P. catenus* (5.22 MPs g^{-1}) but the reason for these high numbers are unclear, it may be possible that by increasing the sample size studied than these numbers may be less of an outlier. (Fig. 5-2).

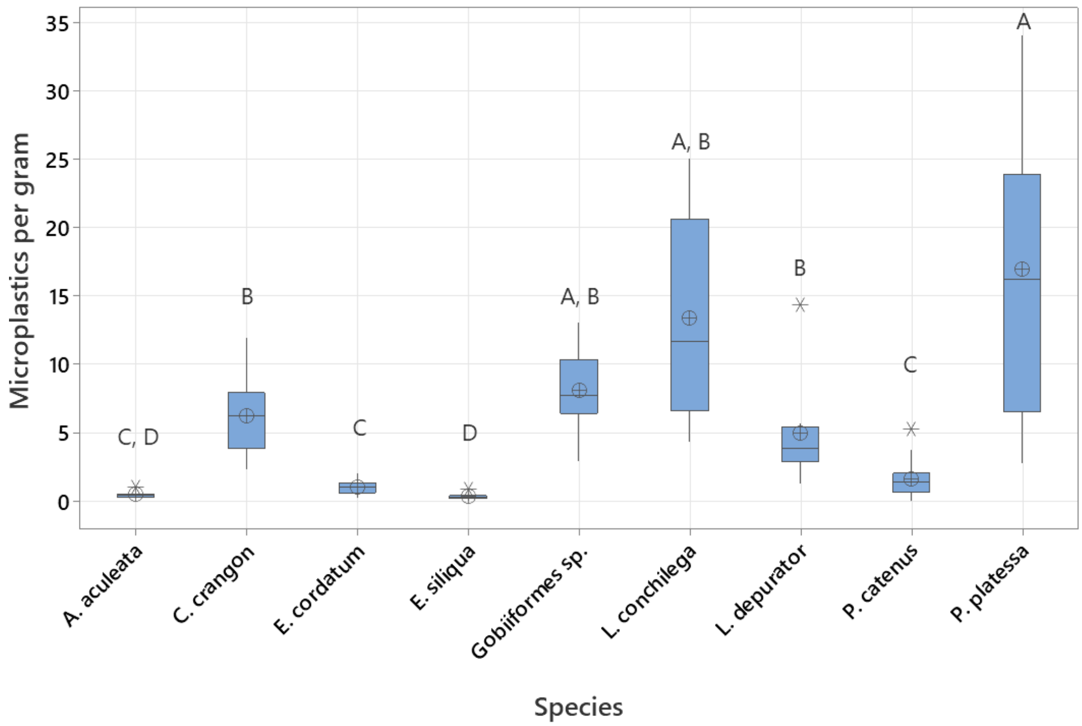


Figure 5-2: Differences in MPs g^{-1} between species. Asterisks represent outliers. Circles with cross interior represent the mean. Solid horizontal lines represent the median. Blue rectangles represent the interquartile range Q1 – Q3 and whiskers represent the range. Species that share a letter do not have significantly different means.

Two sample t-tests carried out for MPs ind^{-1} when assessing habitats found that there was no statistically significant difference between the subtidal and intertidal community (P value = 0.386), however, this was not the case when assessing concentrations (MPs g^{-1}) and the habitats exhibited statistically significant differences in this regard with intertidal values being greater (P = 0.000) (Fig. 5-3).

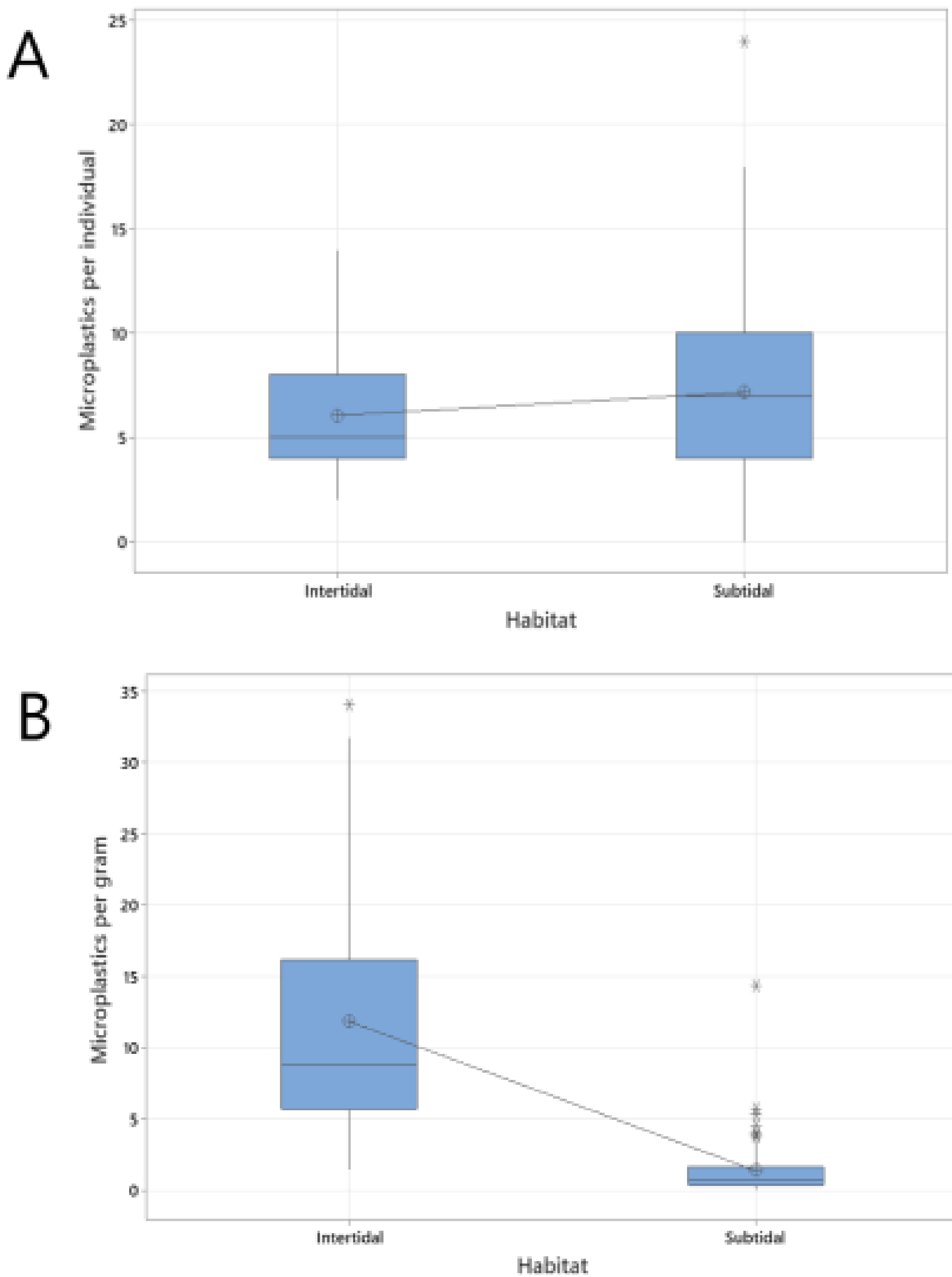


Figure 5-3: Differences of microplastics abundances between habitat type for MPs ind^{-1} (A) and MPs g^{-1} (B).

Specimens recovered from the subtidal habitat generally possessed higher MPs ind^{-1}, (range of; 5.55 – 11 MPs individual^{-1}, average; 7.11 ± 4.72 MPs ind^{-1}) (n = 76). Specimens examined from the intertidal habitat (n = 56) had a range of 5.58 – 6.42

MPs individual^{-1} and had an average of 6.11 ± 3.1 MPs individual^{-1} including *L. conchilega* casings. The casings of *L. conchilega* possessed 1.37 microplastics cm^{-1}. One-way ANOVA analysis and Tukey's post-hoc analysis determined that MPs ind^{-1} were more closely related between species than MPs g^{-1} with the mean values of *P. catenus* were significantly different to those in *L. depurator* and *A. aculeata*. (Fig. 5-4). One specimen of *P. catenus* had 24 microplastics present in its tissue mainly blue fragments and it may be possible that these resulted from a larger particle that fragmented during sample processing (Fig. 5-4). .

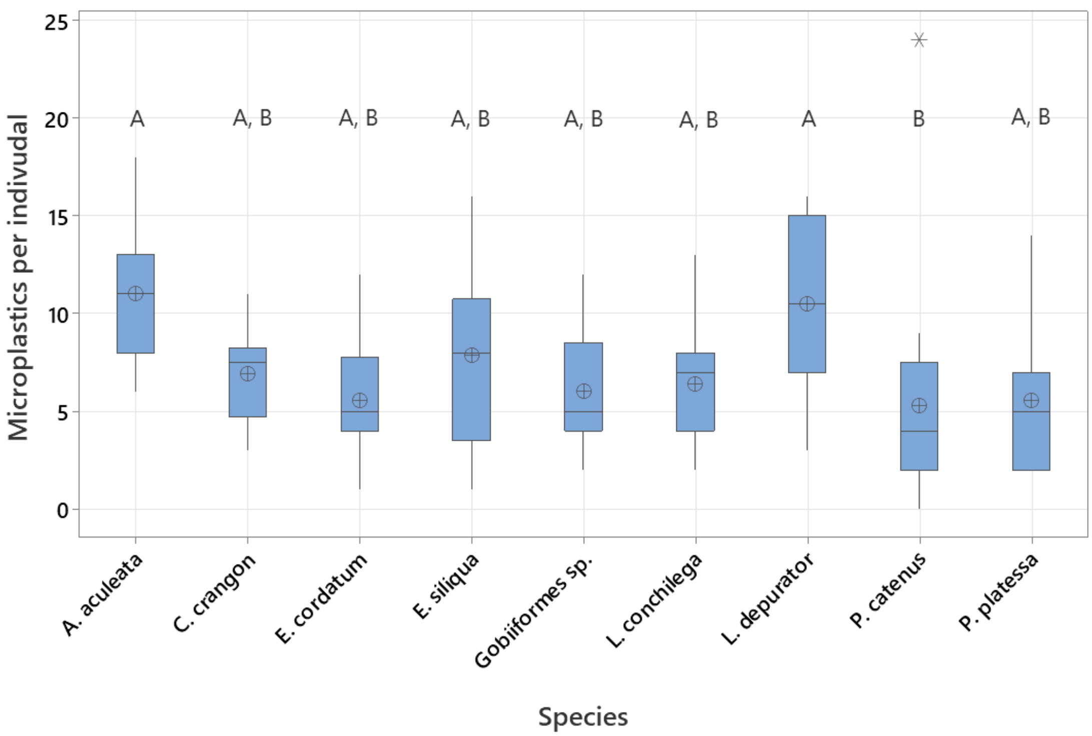


Figure 5-4: Differences in MPs ind^{-1} between species sampled from both subtidal and intertidal habitats studied.

Opportunistic feeding species / scavengers in this study (*L. depurator, A. aculeata*) had significantly greater abundances of MPs ind^{-1} than active predators or the deposit feeding *E. cordatum* but similar to those found in the filter feeder, *E. siliqua*. In terms of MPs g^{-1} there was statistically significant greater microplastic concentrations in active predators than the other three groups who were statitstically similar to each other (Fig. 5-5).

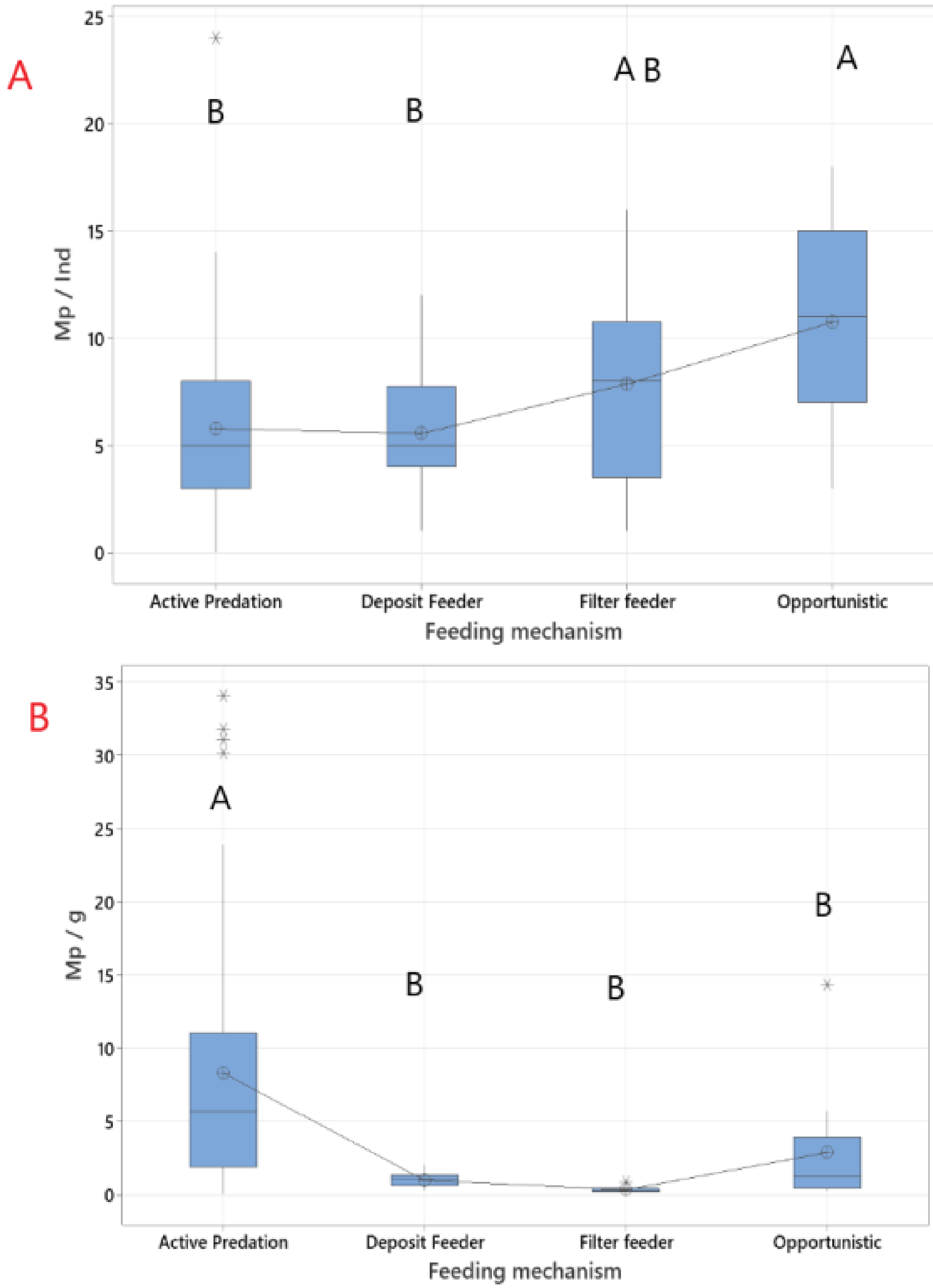


Figure 5-5: Microplastic levels between feeding mechanisms, A: MPs ind^{-1} per individual. B: MPs g^{-1}.

Despite differences in microplastic concentrations between species and in the total recovered from each habitat similar microplastic shape profiles were found in both sets of species from the intertidal and benthic habitats when assessing them on a habitat-basis which was not evident when examining species alone (Fig. 5-6). From subtidal species fibres accounted for the majority of microplastics found in those examined (58.9%), which was followed by fragments (27%), films (13.9%) and beads (0.2%). A similar trend was noted in the microplastics from the species in the intertidal habitat with fibres the majority of those recovered (60.9%) this was followed by fragments (29.6%), films (8.7%) and beads (0.8%). Microfibres were recovered from the tubes of *L. conchilega* with the greatest frequency accounting for 90.9% of those recovered which was followed by fragments (6.5%) and films (2.6%). Microfibres were the dominant shape found in the 4 species studied in the intertidal zone accounting for a range of: 55% in *P. platessa* to 91% of those found in the casings of *L. conchilega*. Of the five species recovered from the subtidal habitat microfibres were the dominant microplastic shape found in 4 ranging from: 49% in *E. siliqua* to 82% in *L. depurator*. Fragments made up the largest portion of microplastic recovered in *P. catenus* (51.4%) followed by fibres (43.2%). Fragments made up the 2nd largest portion of microplastics in 8 species studied, the aforementioned gastropod, *P. catenus* being the exception.

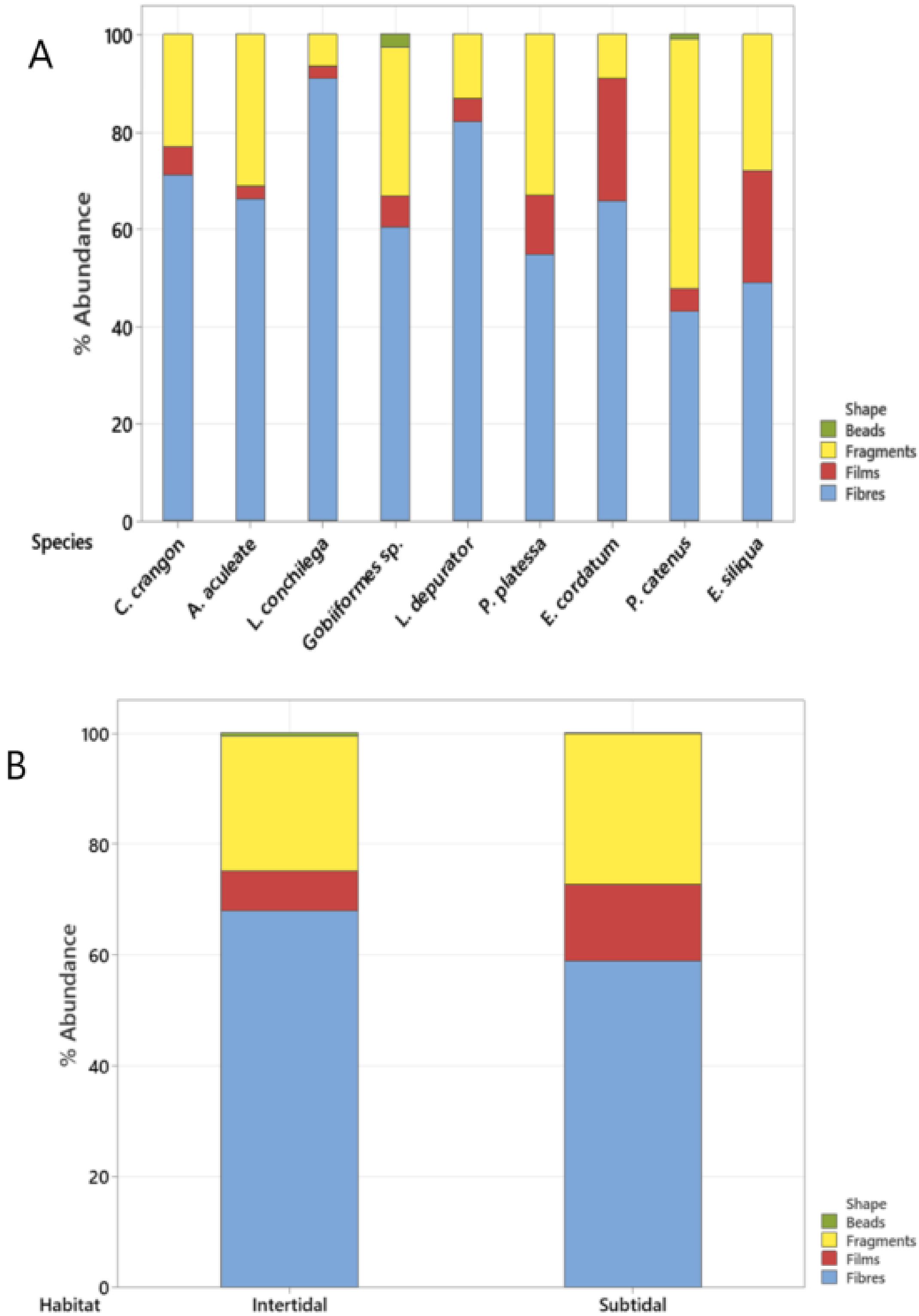


Figure 5-6: Microplastic shape compositon for individual species (A) and between habitats (B).

With regards to size composition of microplastics recovered, this varied on a species level (Fig. 5-7). Larger microplastics in the size range 1-5mm accounted for a range of 21.6% (*E. cordatum*) to 76.6% (*L. conchilega*) of those recovered per species. The predator, *P. catenus*, had the largest portion of smaller-sized microplastics <300μm of species studied accounting for 57.7% of those recovered while the casings of *L. conchilega* had the smallest portion, 10.2%. For subtidal and intertidal specimens, the majority of microplastics recovered were <1mm in size, accounting for 70.7% and 53.0% of those recovered respectively.

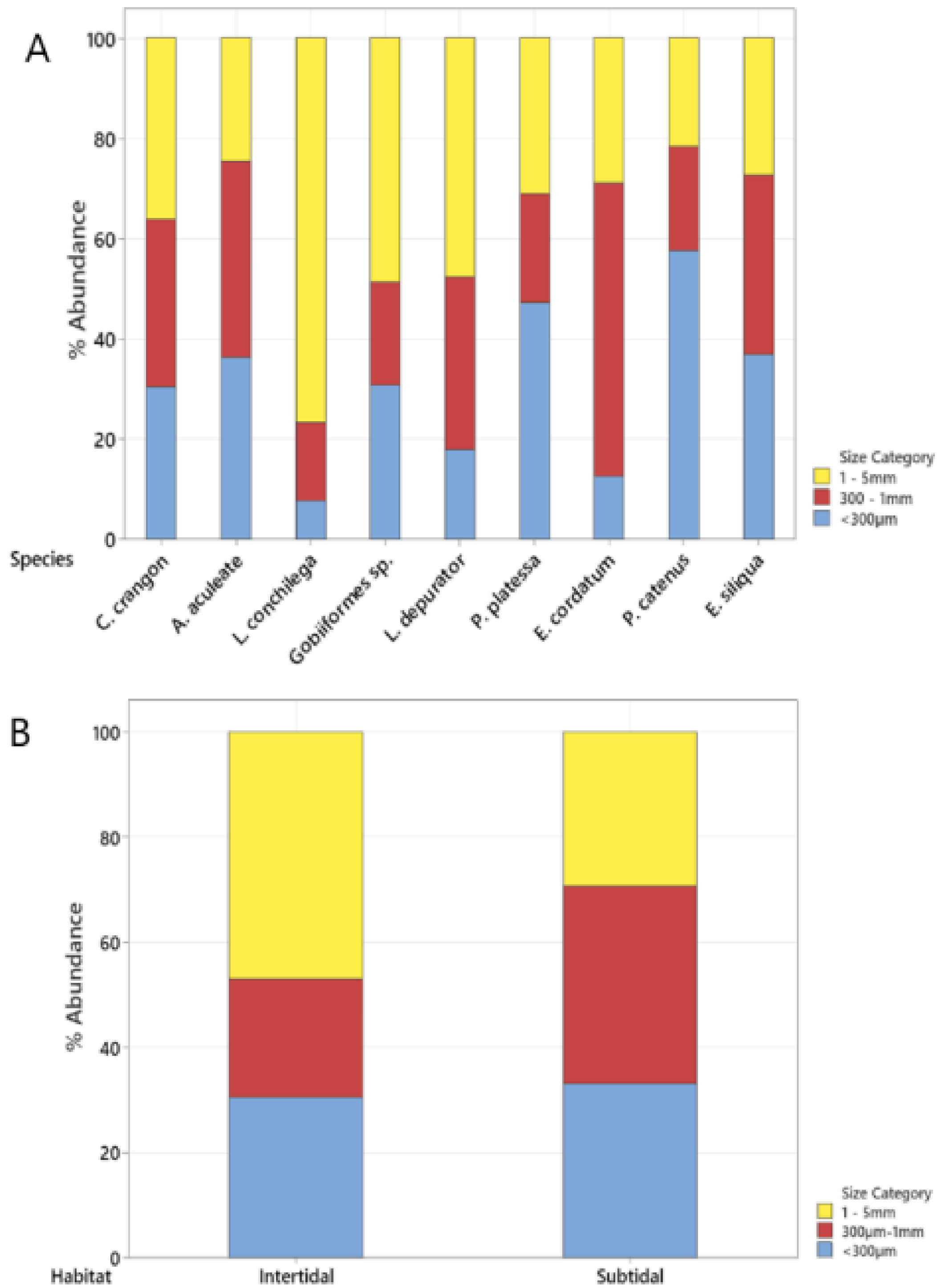


Figure 5-7: Size breakdown of microplastics recovered for individual species (A) and on a habitat basis (B).

Microplastics were found in a variety of colours across the 9 species studied (Fig. 5-8). For species collected via Seine netting from the intertidal zone blue was the dominant colour (41.8%), followed by transparent / white (37%), black (8.8%), other (6.1%) with green and red making up the remaining 6.3%. For subtidal species transparent / white was the most prominent colour (44.3%), followed by blue (25.6%), black (14.3%), red (6.5%), with the remaining 9.3% consisting of green, multi-coloured and other colours. Blue and transparent / white microplastics were the most commonly found across all species studied which was followed by black and red colours. Other colours, green and multi-coloured particles were found with less frequency.

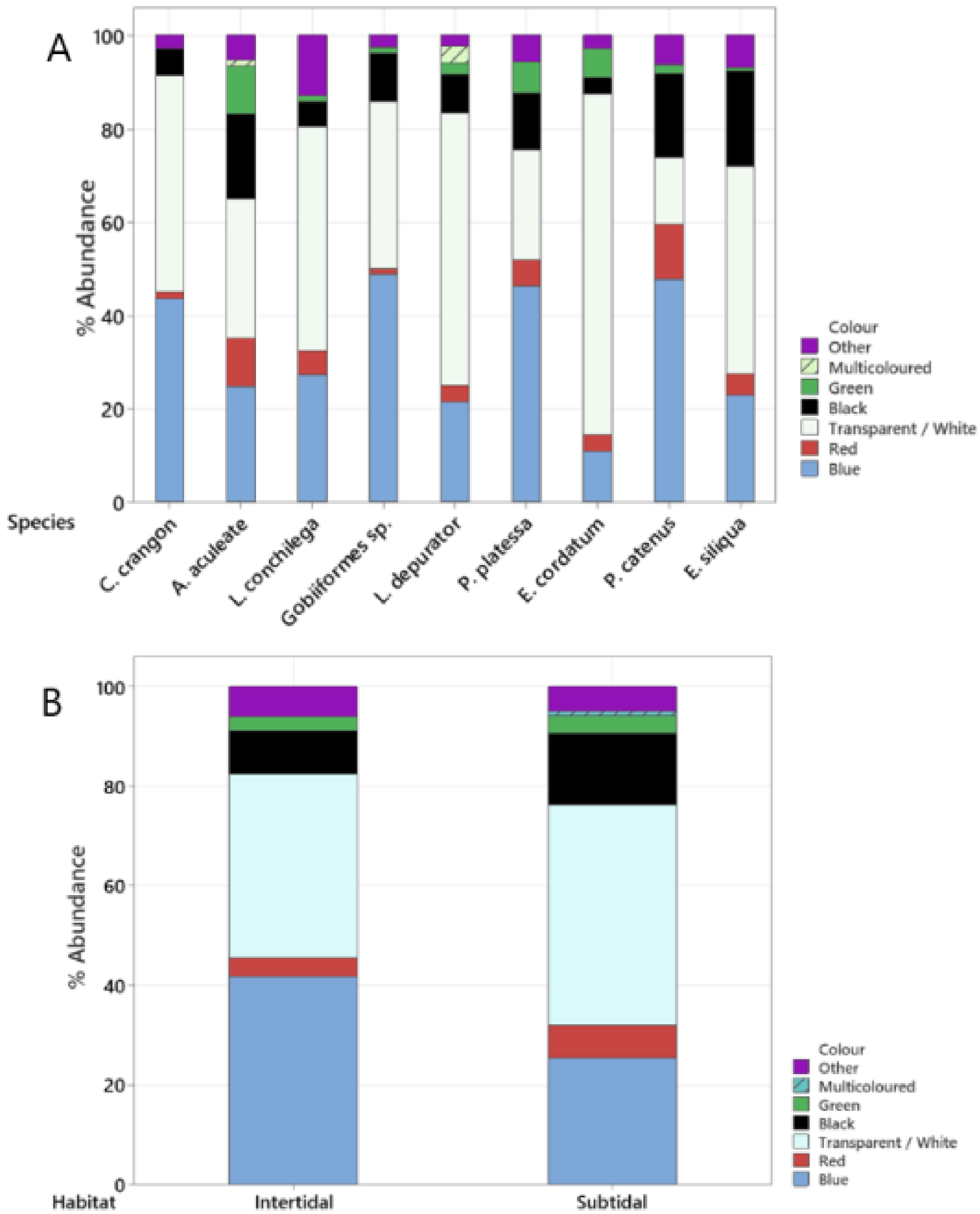


Figure 5-8: Colour composition of microplastics in individual species (A) and based on habitat (B).

5.4.2 Raman analysis results

A subsample of suspected microplastics was selected for Raman analysis (n = 125, 14.3%) and matches were identified for 95 microplastics. The majority of microplastics (64%) identified were polyamides (PA) while Polyvinylchloride (PVC), polyesters and other polymers made up 23% (Fig 5-9). One fibre was identified as polyethylene and this was the only particle recovered with a density lower than that of seawater. Unspecified polymers (described in 2.9.2) were also noticed. The greatest range of polymers that were positively identified were found in the filter feeder, *E. siliqua* (polybutylene (PBT), poly(tetramethylene terephthalate)–poly(tetramethylene ether (PBT-PTMG), PA, PVC and an ethylene copolymer) while *gobiiformes* sp. and *P. platessa* had three different polymers present in their tissues (Fig. 5-10). Some examples of identified microplastic polymers are displayed in figure 5-11.

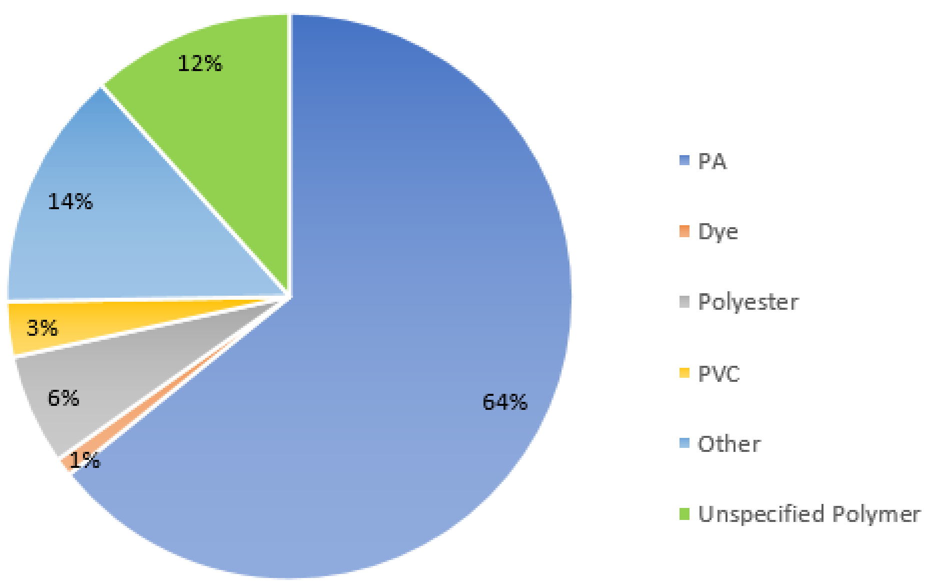


Figure 5-9: Polymer composition of microplastics recovered from biota samples.

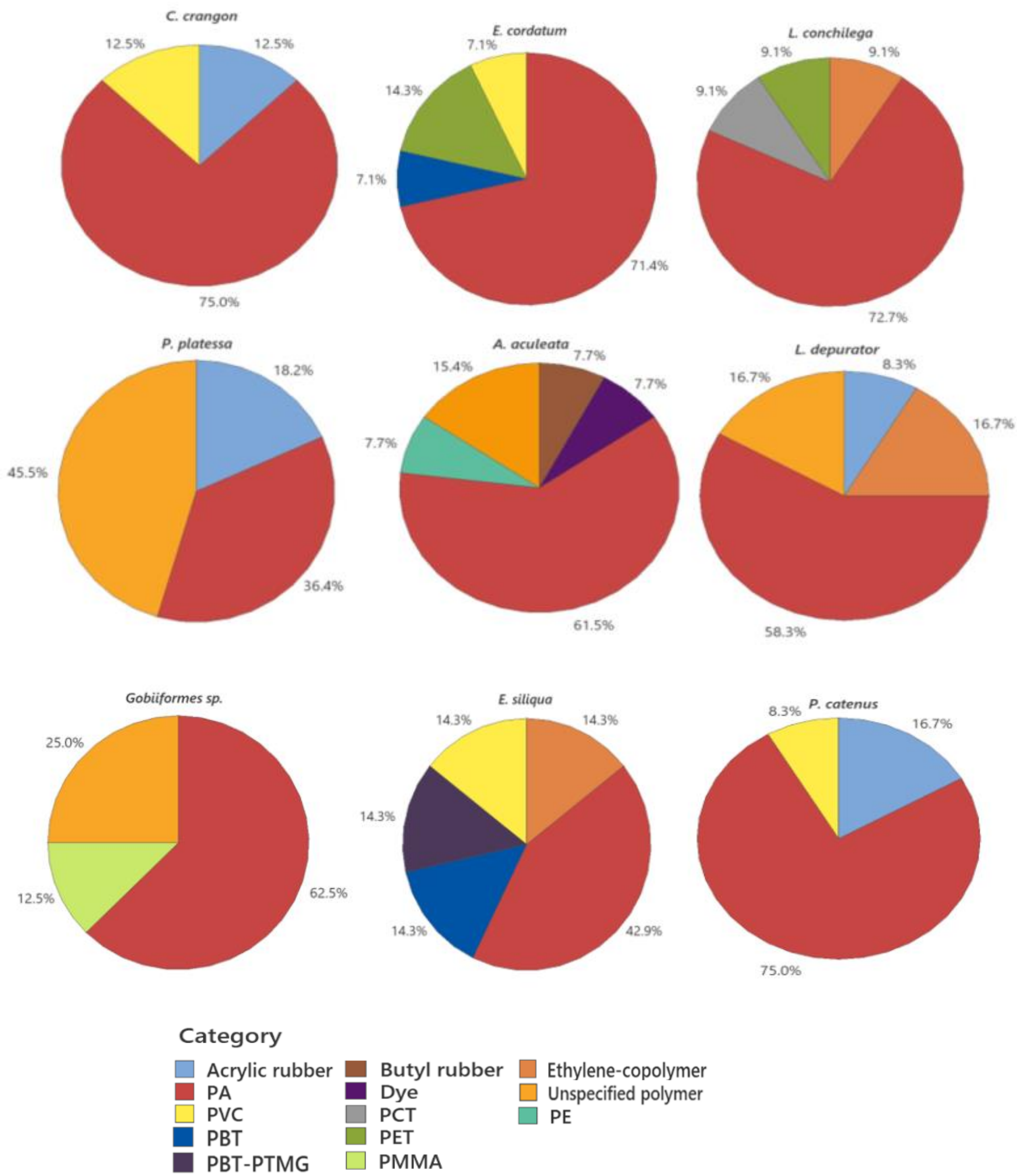


Figure 5-10: Microplastic composition recovered in individual species.

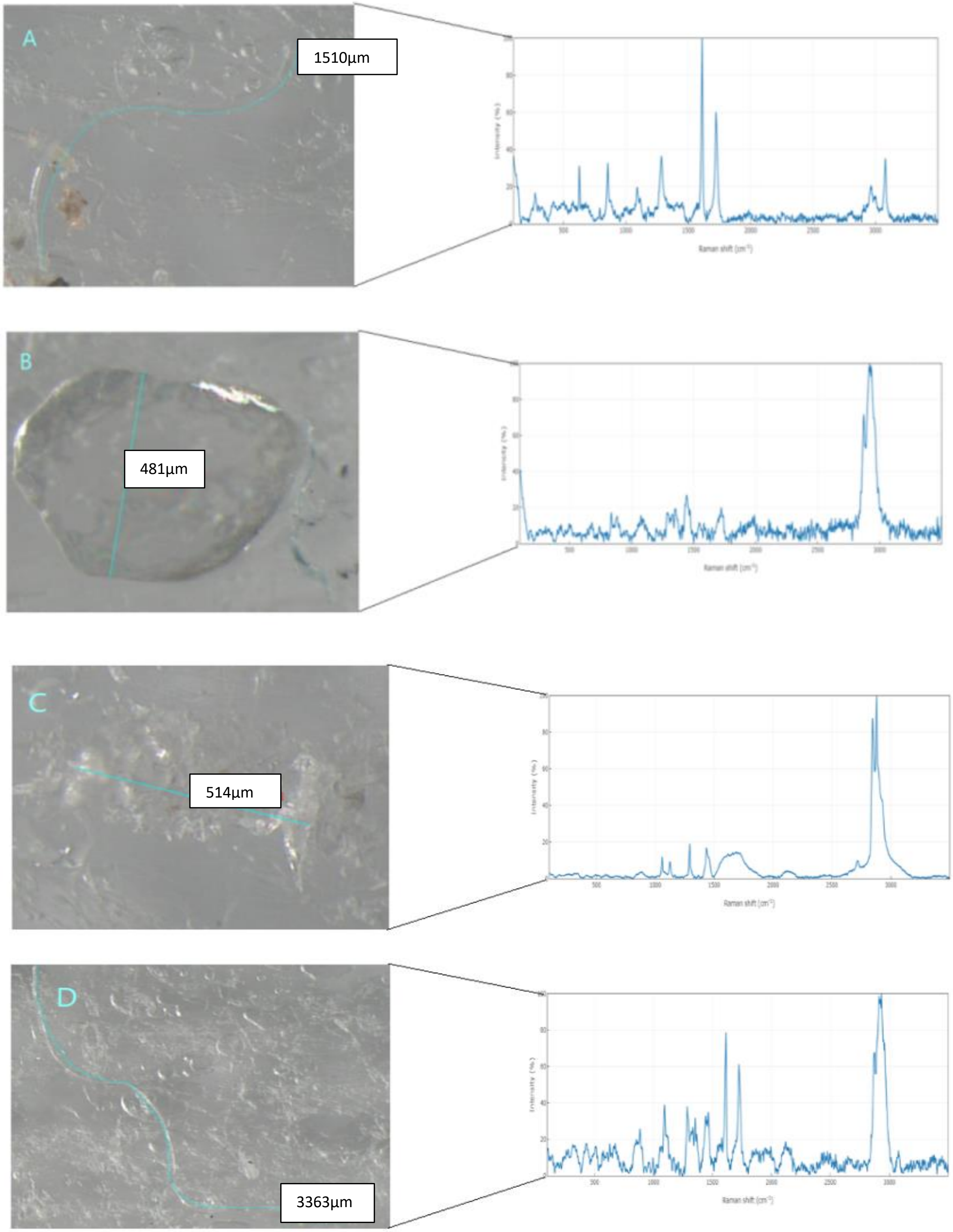


Figure 5-11: Microplastics identified in marine organisms. A: transparent polyester fibre from *E. cordatum*. B: Transparent polyamide film from a tube of *L. conchilega*.

C: transparent polyethylene warped film from *A. aculeata*. D: transparent PBT-PTMG fibre from *E. siliqua*.

Linear regression analysis was conducted for weight versus the number of microplastics present in all individual species. However, no strong trends were identified indicating that weight was not a good predictor for microplastics consumed or incorporated into mason worm casings. An improved fit was found for *Gobiiformes* sp. when using a polynomial trendline (R^2 = 0.3966) (linear: R^2 = 0.2983) which was the best of all species examined indicating a fluctuating trend for this species. Length was examined as a predicator for micro particle abundance for the fish; *Gobiiformes* sp., *P. platessa*, the bivalve; *E. siliqua* and the casings of *L. conchilega*. Neither linear nor polynomial regression analysis indicated that length was a good predictor of micro particle abundance in the aforementioned species with *Gobiiformes* sp. presenting the best relationship for both (linear: R^2 = 0.5699, polynomial: R^2 = 0.7207) (Fig. 5-12).

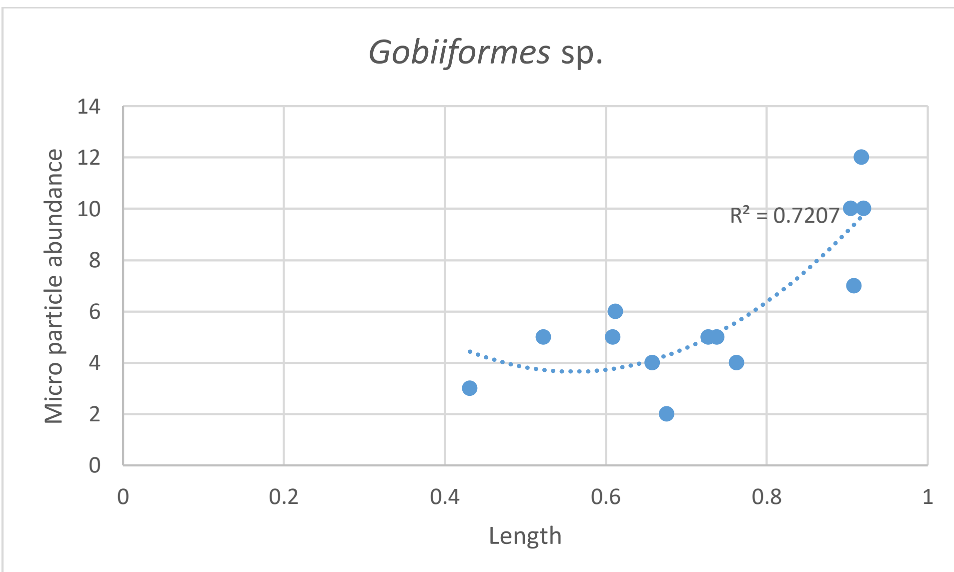


Figure 5-12: Relationship between fish length and microplastic abundance in *Gobiidae* sp.

5.5 Discussion

Previous studies on marine species have idenitified microplastics in species collected from deeper waters around Ireland (Joyce *et al*. 2022; Pagter *et al*. 2021; Pagter *et al*. 2020b). To the best of the authors' knowledge this study is the first to examine species in shallower habitats on the eastern Irish coast. Further comparisons to other studies on species in shallow marine environments are displayed in table 5-2 below.

Table 5-2: Microplastics recovered in species from other near-shore / shallow environments compared to the results of this study.

Species	Location	Depth / Sampling Method where unavailable	MPs Ind^{-1}	MPs g^{-1}	Main Polymers recorded	Main Polymers recorded
P. platessa, C. crangon, L. conchilega, gobiiformes sp.	Dundalk Bay, East-Coast Ireland (Inner-bay)	<1m	Average 6.11 ± 3.11	Average 12.02 ± 8.34	PA, polyester, unspecified polymer, others	Current Study
Subtidal Invertebrates	Dundalk Bay, East-Coast Ireland (Outer-bay)	2-4m	Average 7.11 ± 4.72	Average 1.36 ± 1.94	PA, Polyesters PVC, unspecified polymer, others	Current Study
Twelve invertebrate species	Terra Nova Bay, Antartica	25-140m	Average 1.0 items individual^{-1}	.7 items mg^{-1} dw	PA, PE	Sfriso *et al*. (2020)
Five invertebrate phyla	Galway Bay, West Coast of Ireland	15–91 m	0.79 ± 1.14 particles individual^{-}	N/A	Cellulose, Polyvinyl acetate (PVA)	Pagter *et al*. (2021)
Bivalves, gastropods and crabs	Soft shores in Hong Kong	Intertidal digging and hand collection	0 to 18.4 particles individual^{-1}	0 to 9.68 particles g^{-}	Cellulose, cellophane, PET, PA.	Xu *et al*. (2020)

Three-spined stickleback *Gasterosteus aculeatus*)	Northern Baltic Sea, Finland	Beach Seine Netting	0.2 ± 0.6	N/A	N/A	Sainio *et al*. (2021)
Bleak (*Alburnus alburnus*)			0.2 ± 0.5			
Perch (*Perca fluviatilis*)			0.08 ± 0.3			
Roach (*Rutilus rutilus*)			0.03 ± 0.3			
Five fish species	Charleston Harbour, Southeastern Atlantic coast of the United States of America	Trammel and Seine Netting	average of 27 microplastics per individual	6 microplastics per gram of fish. 21 microplastics per gram of gut.	low-density polyethylene, ethylene propylene diene, polypropylene	Parker *et al*. (2020)
Common periwinkle, *Litt orina littorea*	Galway Bay, Ireland	Hand collection from rocky intertidal shores	0.59 ± 0.90–2.40 ± 2.11 MPs/individual	Average 2.14 MPs/gram	PS, polycarbonate, po lytetrafluoroethylene, PVC, PES, PE, nylon 6 (PA), and viscose	Doyle *et al*. (2019)

The dominance of microfibres in both subtidal and intertidal communities is in line with other studies assessing microplastic pollution of marine communities. Pagter *et al*. (2021) found that 98% of microplastics recovered from benthic communities off the west coast of Ireland were microfibres, while 88% of microplastics found in the digestive tract contents of four fish species from the north-west Iberian shelf were microfibres (Filgueiras *et al*. 2021). Microfibres also consisted of 80% of microplastics found in fish, 78% of those found in shrimp and 78% of those found in mussel species sampled from the Gorgan Bay in the Caspian sea (Bagheri *et al* 2020). Microfibres also were the dominant shape found in the intertidal gastropod, *Littorina littorea,* sampled on the west coast of Ireland, consisting of 97% of recovered microplastics (Doyle *et al*. 2019). Furthermore, microfibres dominated the morphology of microplastics recovered in the other environmental matrices examined in the course of this thesis.

The high proportion of blue and transparent/white microplastics found in both communities is similar to other studies that reported colours in microplastics from marine species. Pagter *et al*. (2021) found that 77% of microfibres were blue and 8% transparent found in the benthic communities off the west coast of Ireland. Blue was also the prominent colour found in *Nephrops norvegicus* in fishing grounds around Ireland (Joyce *et al*. 2022; Hara *et al*. 2020) and off/white-clear were the dominant colour found in four species of coastal fish in the northern Baltic Sea on the coast of Finland (Sainio *et al*. 2021). Blue microplastics were also the dominant colour recorded in a review of 132 articles on marine vertebrates (Ugwu *et al*. 2021). Blue microfibres were the dominant type found in commercial species and bycatch in areas of the Southern Baltic Sea which was linked to fish acquisition i.e., through nets used for trawling (Piskuła and Astel, 2023). Fibres in the marine environment have also been linked to the fragmentation of fishing ropes, nets and lines in the past (Koongolla *et al*. 2020). Blue and transparent microfibres were also the main profile of microplastic found in *C. edule* examined in Dundalk Bay over four sampling seasons (Chapter 4). The presence of microfibres in species found in Dundalk Bay is of concern given recent laboratory work conducted on estuarine species. Siddiqui *et al*. (2023) found that larval mysids and juvenile fish both exhibited behavioural impacts following exposure to polyester and polypropylene microfibres and that these impacts were amplified in treatments with lower salinities, additionally, growth for both species was affected in at least one of three tested salinities at microfibre concentrations as low as 3 particles/ml. Given that juveniles of both *P. platessa* and *C. crangon* in this study possessed comparatively high levels of microfibres, it is possible that they are also experiencing illicit affects both in terms of growth and behaviour due to this, however, an in-depth laboratory experiment is needed to confirm this theory.

In terms of size of microplastics recorded, the majority found in organisms from both habitats were less than 1mm in size which is in keeping with other studies (Pagter *et al*. 2020b; Fang *et al*. 2020; Zhang *et al*. 2020). There was a greater percentage of microplastics larger than 1mm in size recorded in intertidal species than in subtidal ones. This may be due to the proximity to shore of this environment and that microplastics may be entering the bay from terrestrial sources and may be relatively 'new' before they breakdown to smaller sizes, especially given that these specimens were smaller than those recovered from the subtidal habitat studied.

In line with the findings of Foekema *et al*. (2013), Güven *et al*. (2017), Sainio *et al*. (2021) and Pagter *et al*. (2020b) there was no strong relationship between the size of the study specimen - either weight or length - and the number of microplastics present in the soft tissue examined. However, the high frequency of occurrence amongst study specimens may mean that increased damage of the digestive tract especially in the smaller / juvenile specimens found in the intertidal habitat may be occurring. There was a higher occurrence of microplastics in the Dundalk Bay specimens than in other recent studies of marine species. Microplastics were found in 9% of small coastal fish in the Northern Baltic Sea by Sainio *et al*. (2021) while 27.5% of fish caught within the urban area of Helsinki had plastic occurrence. Pagter *et al*. (2021) reported an incidence rate of 48.5% of invertebrates sampled within the infaunal benthic community on the west coast of Ireland. Interestingly, a high frequency occurrence (83%) was also noted in benthic invertebrates sampled from the remote region of Terra Nova Bay in the Ross Sea of Antarctica (Sfriso *et al*. 2020). Microplastics, however, were present in 99% of 5 fish species examined in an urbanised bay environment on the Southeastern Atlantic Ocean coast of the United States (Parker *et al*. 2020). Furthermore, 100% of juveniles of the patagonic silverside fish *Odontesthes* sp. sampled from shallow coastal waters contained microplastics (Mendoza *et al*. 2022). The presence of microplastics in juvenile *P. platessa* found in this study is particularly troubling as smaller sized gastro-intestinal tracts are more likely to sustain damage from microplastics than adult fish. Microplastic fibres fed to juveniles of *Lates calcarifer* led to an altering of gut microbiome community composition and several species beneficial for host were inhibited (Xie *et al*. 2021). Fibres (2-3mm) although they could be eliminated effectively were also shown to cause oxidative stress in juveniles in the same study (Xie *et al*. 2021).

The high occurrence of microplastics in samples taken from Dundalk Bay when compared to other studies may be due to a number of factors. The shallow nature of Dundalk Bay may lead to an increased concentration of microplastics present in the waters here when compared to deeper more dynamic bays. At low tide much of the bay is exposed as freshwater inputs dominate which have been shown to transport microplastics in chapter 3 of this thesis. Previous work has shown that microplastics can be diluted depending on the size of the aquatic environment (Barrows *et al*. 2018). This would indicate that although more microplastics may be entering deeper coastal environments their concentration in the water column gets reduced and therefore the likelihood to be ingested or inhaled by an organism also does.

Fish specimens examined in this study were digested whole due to their small size. While, this type of whole-body digestion is only practical for smaller sized specimens it does, however, provide realistic environmental data in terms of trophic transfer as the whole organism will generally be consumed by predators leading to a total transfer of all microplastics present in the prey species. There is growing evidence that microplastics can get transferred in the food chain (Nelms *et al*. 2018; Farrell and Nelson, 2013) raising concern about detrimental implications for bioaccumulation from one trophic level to the next.

Excluding the casings of the *L. conchilega*, the three species with the highest microplastic concentrations were the intertidal fish *Gobiformes* sp. and juvenile *P. platessa* as well as the brown shrimp *C. crangon*, which may reflect their overlapping feeding behaviours. This is especially true between *C. crangon* and juvenile *P. platessa* as there can be considerable diet overlap between these two species later in the summer (Evans, 1983) and all three species behave as generalists with respect to taking food items (Evans and Tallmark, 1979, 1984; Evans 1983). The statistically higher concentrations of microplastics found in species from the intertidal habitat in this study may be due to the proximity of this area to the terrestrial environment which is the main source of plastic litter entering the marine environment (Sheavly and Register, 2007). Intertidal sediment has previously been shown to possess greater microplastic abundances than subtidal sediment (Markic *et al*. 2023). In the Lagoon of Venice microplastic concentrations of microplastics were higher in the inner part of the lagoon, where water currents were low (Vianello *et al*. 2013) and upper intertidal areas showed higher microplastic abundance in comparison to lower intertidal zones in Atlantic Argentinean estuaries (Díaz-Jaramillo *et al*. 2021). Intertidal sediment found in Dundalk Bay exhibited twice the estimated safe level of microplastic loadings as shown in chapter 4 of this thesis (Everaert *et al*. 2018).

Interestingly the infaunal species (*A. aculeata*, *E. siliqua*, *E. cordatum*, *P. catenus*) studied had the lowest concentrations (MPs g^{-1}) present and this may reflect decreased levels of microplastics sinking down to the subtidal areas in this bay environment and thus being ingested by these species. Another potential reason for this difference may be linked to their respective mobility / emergence behaviours. More mobile species studied had greater microplastic concentrations present than more sessile organisms. The crab, *L. depurator*, which is the most mobile organism collected from the subtidal zone had the highest microplastic concentrations and similarly to the snail, *P. catenus* can actively forage in shallower waters which followed the crab. Notably, some species of marine snail are known to forage in the intertidal zone (Hayford *et al*. 2021). In terms of microplastic concentration abundance the next most mobile subtidal organism studied was *A. aculeate* which has been noted to only take prey items when it is buried (Mettam, 1980). The least mobile species analysed in this study (*E. siliqua*, *E. cordatum*) also had the lowest concentrations of microplastics recorded. The razor clam, *E. siliqua*, sampled from the North-West Mediterranean Sea reported higher contamination levels than those reported in this study both in terms of MPs g^{-1} and MPs ind^{-1} with values of 2.45 ± 2.59 MPs g^{-1} and 12.5 ± 13.2 MPs indiviudal^{-1} respectively (Expósito *et al*. 2022). In keeping with the findings of this study Bour *et al*. (2018) noted that filter feeders also had the lowest levels of microplastics when compared to deposit feeders and predators in Oslofjord.

The low numbers of microplastics reported in the filter-feeding bivalve in this study when compared to other study species underline that it may be unsuitable as a biological indicator for monitoring microplastics in coastal environments even as other bivalve species e.g., *Mytilus edilus* are considered suitable (Beyer *et al*. 2017). This in turn, may have attributed to low microplastic concentrations in *L. depurator* compared to intertidal species as they can feed on exposed *E. siliqua* following bed disturbance (Fraser *et al*. 2018). Filter feeders are often presented as the ideal biomonitor for microplastic pollution given the high volumes of water they process (Walkinshaw *et al*. 2020). However, a recent review of microplastic contamination in benthic invertebrates found that they are not more prone to microplastic uptake than other species which was attributed as likely due mechanistic differences between species that alter microplastic capture and retention rates relative to other groups (Porter *et al*. 2023). The findings of this study support those observed in marine benthic invertebrates as whole.

Doyle *et al*. (2019) reported that microplastics present in intertidal gastropods collected on the West Coast of Ireland were composed mainly of fibres (97%) with fragments consisting of only 3%. Concentrations of microplastics in the gastropod *P. catenus* in this study were also lower than those reported in the study by Doyle *et al*. (2019) on *Littorina littorea,* which have values of 2.14 MPs g^{-1} compared to 1.6 ± 1.3 MPs g^{-1} which was possibly linked to their feeding on fucoid algae that may trap and hold MPs (Gutow *et al*. 2015). This difference may also be linked to their feeding in habitats closer to the terrestrial environment which increase microplastic exposure.

The high proportion of PA recovered in specimens of this study is not surprising given that polyamides constitute about 44.7% of polymers discharged in the marine environment (Mejías *et al*. 2023, Hamidian *et al*. 2021, Mofakhami *et al*. 2020, Wang *et al*. 2018). The presence of only one microplastic with a density less than seawater identified in specimens would indicate that buoyant microplastics are remaining in the surface water in Dundalk Bay and thus not being ingested or interacting with epifaunal or infaunal species that were the subject of this study.

Denser microplastics also dominated those found in marine sediment examined in chapter 4 of this thesis. Buoyant microplastics may float off-shore and their density may increase due to a variety of processes, notably polyethylene has been documented in seawater at a depth of 2200m at Rockall Trough in the North Atlantic Ocean (Courtene-Jones *et al*. 2017). PA (nylon) also made up 86% of microplastics recovered in twelve invertebrate species sampled from the Ross Sea in Antarctica (Sfriso *et al*. 2020).

The high occurrence of PA detected from specimens in Dundalk Bay is particularly worrying. Recent work has displayed that in real water matrices the chemicals parabens can adsorb to PA which were noted to have potentially important environmental implications since it may alter bioavailability of contaminants, environmental fate, and biomagnification and bioaccumulation of pollutants, as well as toxicity to biota (Mejías *et al*. 2023). It can be difficult to establish links between polymer characterisation and anthropogenic uses as polymers can be attributed to a wide range of sources (Carr, 2017). However, PA, polyester and PVC are commonly used in textiles and the abundance of microfibres in species studied as part of this work indicate that WWTPs located close to the coastline may be emitting these types of microplastics into Dundalk Bay. Previous studies have documented the presence of microplastics in the effluent of WWTPs (Liu *et al*. 2019; Murphy *et al*. 2016). Additionally, domestic washing of textiles can release microfibres into the environment (Šaravanja *et al*. 2022; Ziajahromi *et al*. 2016).

Fishing gear consists of various polymer types, including PA, PP and PE (Nelms, *et al*. 2021). Significant microplastics can be generated from Abandoned, Lost, and Discarded Fishing Gear. A study on ALDFG in Southern England found rope and nets found on beaches had the potential to generate 1277 ± 431 microplastic pieces m^{-1} (Wright *et al*. 2021). It must be noted that not all fishing plastics such as ropes generate microfibres though use. Napper *et al*. (2022) demonstrated that the abrasion of ropes used on board fishing vessels shed irregularly shaped and fragmented rather than fibrous microplastics and that fragments found in marine environments may have been misattributed to terrestrial sources. Given the fishing pressures exerted on Dundalk Bay it is likely that significant microplastics stem both from fishing plastics in use and ALDFG on beaches in the area, however, it is unclear the proportion that stems these sources.

The potential transfer of microplastics between prey and predators is unclear and likely varies between species. Growing evidence points towards the possibility that microplastics can get transferred in the food chain (Farrell and Nelson, 2013, Nelms *et al*. 2018) which raises the concern for detrimental impacts due to biomagnification in higher species. However, Walkinshaw *et al*. (2020) assessed that microplastics do not biomagnify as feared and instead organisms towards the base of the food chain at lower trophic levels are more contaminated with microplastics potentially posing a greater threat to their health.

Regardless of the prospect of biomagnification in marine food webs, the high prevalence of microplastics in organisms examined in this study, particularly in juveniles of the commercially important *P. platessa* is of concern as they may experience detrimental impacts such as mechanical interference in their feeding and digestion processes than larger sized fishes (Jovanović, 2017). The average number of MPs ind^{-1} for *P. platessa* (5.58) found in this study was higher than other studies examining microplastics in fish including those on the same species, 1.46 (Welden *et al*. 2018) and 0.9 ± 1.79 reported by Murphy *et al*. (2017), and to that observed in demersal fish studied by Lusher *et al*. (2013) (1.2 ± 0.54).

Filter feeders *(E. siliqua*) in this study displayed on average values (7.85 MP ind^{-1}) between opportunistic feeders / scavengers (10.73 MP ind^{-1}) and active predators (5.78 MP ind^{-1}) and were statistically similar to all other feeding mechanism. Additionally, they reported the largest spread of polymers in their soft tissue and may have potential as bioindicators of polymers present in an environment although they presented the lowest MP g^{-1} values of any species present. As filter feeders, it has been noted that any differences in microplastic ingestion are likely due to microplastic distribution in their habitat (Walkinshaw *et al*. 2020). Piarulli *et al*. (2020) noted that suspension and facultative deposit feeding bivalves had a lower microplastic occurrence (0.5% to 3%) than omnivores (95%) but contained a much more variable distribution of microplastics. The presence of microfibres in wild-caught *L. depurator* in this study lends to the possibility of impacts on this species as the crab, *Carcinus maenas* showed a reduction in food consumption and energy available for growth after ingestion of (PP) rope fibres (Watts *et al*. 2015). The presence of microplastics in the urchin, *E. cordatum* in this study may indicate a microplastic burial below the seabed surface similarly to that carried out in brittlestars (Coppock *et al*. 2021) both by passively transporting downwards when maintaining their burrows but also through ingestion and egestion. When present in significant numbers this species could lead to increased microplastic sequestration in marine environments, however, a more detailed study would be needed to test this theory.

5.6 Conclusion

The results of this study present a first look at microplastic pollution of the subtidal and intertidal communities in Dundalk Bay. The ubiquitous presence of microplastics found in both groups of species in this Bay is evidence of the anthropogenic pressure exerted on the bay. Descriptor 10 of the Marine Strategy Framework Directive (MSFD; 2008/56/EC) states that anthropogenic litter should not negatively impact the environment and microplastics were identified in all subtidal and intertidal species studied as part of this work.

The differences between species and feeding mechanisms in terms of MPs g^{-1} and MPs ind^{-1} reaffirm the need for a multi-species approach for assessing microplastic contamination levels for this shallow bay environment as documented by Pagter *et al*. (2020b) and that studies that only examine one species may be over- or underestimating microplastic pollution levels. The importance of examining Dundalk Bay using an ecosystem-approach are highlighted by the varying concentrations of microplastics found between feeding mechanisms of species examined in this current study. The examination of one species in this environment to assess microplastic levels would have resulted in skewed and underestimating microplastic contamination. For example; by only studying the predatory gastropod, *P. catenus* it would be observed that fragments are the dominant shape of microplastic present, alternatively microplastic presence would have been noted to be very low by only studying the filter-feeding bivalve, *E. siliqua* even as bivalves are considered ideal for microplastic assessments.

The low occurrence of buoyant polymers in species studied coupled with previous work examining microplastics in the sediment of Dundalk Bay indicates that buoyant plastics may for the most part be remaining at the sea surface or potentially floating offshore and sinking down to deeper waters due to changes in their density.

The species selection in this study therefore likely biased the results of this study resulting in this high occurrence of denser plastics being recovered. For deeper marine environments examining surface-dwelling species in conjunction with bottom-dwelling species will likely render a truer picture of the types of polymers present in the marine environment.

For subtidal species microplastic contamination levels were potentially linked to the mobility of the organisms and their foraging activities with the infaunal species containing the lowest concentrations of microplastics while species capable of foraging in shallower intertidal waters had higher concentrations. Excluding the tubes of *L. conchilega* the highest levels of MPs g^{-1} were found in *P. platessa*, *C. crangon* and *gobiformes* of all species studied and may reflect their overlapping feeding mechanisms although the concentrations found in *P. platessa* were significantly greater. While the greatest diversity of microplastics were found in the filter feeder, *E. siliqua*.

This work presents a first examination of the microplastic pollution in the species recovered from two difference habitats present within the SPA and SAC of Dundalk Bay and the ubiquitous presence of microplastics is of concern for the conservation goals of this shallow marine environment given the multiple purposes that it performs. The noted high occurrence of microfibers for the majority of study species is potentially due to inputs from wastewater treatment plants located on the bay and riverine inputs which have been shown to carry microfibres in their surface waters in chapter 3 of this thesis. Microplastics stemming from terrestrial sources may accumulate in this shallow environment leading to increased ingestion by species found here. Previous studies have displayed that many of the lowest occurrences of microplastic have been recorded in fish caught in offshore and mid-water trawls (Welden *et al*. 2018) and these results support that finding. While the physical properties of Dundalk Bay as a shallow marine environment with many freshwater inputs support a productive nursery as well as providing food for the thousands of seabirds that reside in the area and overwinter here, these properties and its proximity to anthropogenic pressures may lead to a heightened threat of microplastics to species residing there.

Chapter 6: Examining awareness, attitudes and behaviours of stakeholders in Irish fishing towards microplastic

This chapter is a reproduction of the following published manuscript (The published version and survey issued is available in appendix D).

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Stephen Kneel[1]*, Caroline Gilleran Stephens[1], Alec Rolston[2] and Suzanne Linnane[1].

[1]Centre for Freshwater and Environmental Studies, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland.

[2]Goyder Institute for Water Research, Adelaide, South Australia, Australia.

*Corresponding author: stephen.kneel@dkit.ie

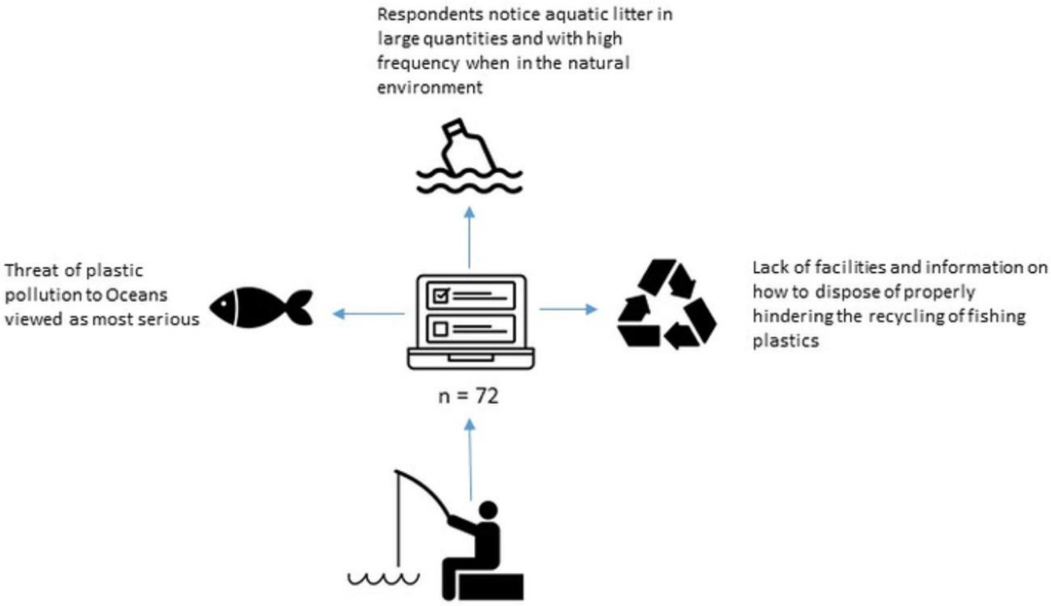

Figure 6-1: Graphical abstract showing main messages from survey respondents.

6.1 Abstract

This paper explores the awareness, knowledge, attitudes and behaviour of members of the Irish fishing community towards environmental topics such as; microplastics, plastic pollution and recycling. We conducted a mixed method survey consisting of 26 questions (2021) involving members of the Irish fishing community (fishers, aquaculturists etc.). Respondents were generally aware of microplastics and the threats they can pose to different environmental matrices. They noticed litter frequently when engaged in their fishing activities (0% never noticed litter) and in large quantities (35% of respondents noticed over 10+ items) but they were likely (likely 40% and highly likely 35%) to remove it from the environment. Durability was the main reason for the selection of most fishing plastics used by respondents (ranked first in 4 of 5 plastic items) while recyclability played a lesser role. Respondents also viewed plastics as cheap and convenient with these terms accounting for 48% of positive connotations related to the word 'plastic', however, in general associated plastic with negative phrases. Barriers to the recycling of used fishing plastics were most frequently identified as being due to a lack of knowledge on how to or a lack of facilities. This study provides novel insight into a previously unstudied cohort in Irish society towards plastics and recycling and can serve as guidance for further work on this group.

Key Words: Aquatic pollution; plastic; microplastic; fishers' behaviour; marine.

6.2 Introduction

Marine litter, especially in plastic form, is a pollutant of increasing environmental concern with around 8 to 10 million metric tonnes entering the ocean annually (Smith and Vignieri, 2021). Ghost-fishing from discarded or abandoned fishing gear (ghost gear) can be dangerous to marine life. For example, 'ghost' nets, can catch a large number of marine organisms attracting other creatures which in turn become entangled in a process known as cyclic catching (Havens *et al*. 2008; Link *et al*. 2019). Plastic's lightweight, durability and resilience to degradation creates ecological issues when released into aquatic environments namely marine wildlife can mistake plastic waste for prey with most then dying of starvation as their stomachs fill with plastic (IUCN, 2022). Macroplastics (>5mm) (SAPEA, 2019) such as nets, bottles and other larger pieces of debris can interact and potentially harm marine life. Microplastics are particles of plastic which have an upper size limit of 5mm (Auta *et al*. 2017; Anderson *et al*. 2016; Li *et al*. 2015). The number of marine organisms that interact with microplastics is likely many times higher than the approximately 700 species that are known to interact with larger marine debris (e.g., through ingestion and entanglement) (Gall and Thompson, 2015; Gregory, 2009). Additionally, microplastics have been shown to be present in commercially important species consumed by humans worldwide such as oysters, mussels, herring, mackerel and tuna etc. (Van Cauwenberghe and Janssen, 2014; Rochman *et al*. 2015) and also in prawns found in Irish fishing grounds (Joyce *et al*. 2022).

The Republic of Ireland (ROI) has a poor record of plastic waste management, rated as the fifth worst country in the European Union (EU) at recycling plastic packaging (Eurostat, 2021). The ROI produces the 2nd most plastic packaging waste in the EU per capita at 224.52kg, much higher than the EU average of 177.24kg per capita with 62.4% recycled (Eurostat, 2021). Furthermore, 91% of studied subtidal and intertidal Special Areas of Conservation (SACs) and Special Protected Areas (SPAs) in the ROI were contaminated with microplastics (Mendes *et al*. 2021).

Plastic waste in the ocean is a major environmental threat and accounts for 85% of marine litter (UN Environmental Programme, 2021). By the year 2050, it is estimated that the mass of plastic debris in the oceans will surpass that of all fish species combined (Jambeck *et al*. 2015). This projection may become reality sooner due to the increased plastic material in circulation as a result of the disposable face masks, components of antigen tests and also increased use of PPE in hospitals from the COVID-19 pandemic (Peng *et al*. 2021). Irish coastal locations that are polluted with more than one disposable face mask has increased from 18% of 710 surveyed locations in 2020 to 21% of the same locations in 2021 (Coastwatch Europe, 2021). Simulations from 24 sampling expedition's estimate that around 268,940 tonnes of floating marine litter are in the ocean ranging from microplastics (<4.75mm) to macroplastics (>200mm) equating to the equivalent of 5.25 trillion pieces of plastic (Eriksen *et al*. 2014). There is a discrepancy between the marine biomes surveyed for marine litter. Most global studies have typically focused on floating debris on the surface layer of the ocean and stranded litter on coastal beach locations (Madricardo *et al*. 2020), while the seabed has been comparatively understudied (Debrot *et al*. 2013; Rizzo *et al*. 2022) in part due to the difficulty in accessing this environment. Some areas of seafloor around Europe's coasts can have up to 10,000 plastic items per hectare (Thompson *et al*. 2009), suggesting a less tangible but still very real threat to fisheries from plastic litter. This is particularly problematic given that microplastics have been documented in shellfishes sold for human consumption (Daniel *et al*. 2021).

The economic cost of marine plastic litter to nearly all marine ecosystem services should not be underestimated. Using 2007 US$ values Beaumont *et al*. (2019) estimated economic costs per tonne of plastic litter in the ocean to services such as fisheries, recreation and heritage were estimated as $3300-$33,000. Furthermore, early studies of commercial and subsistence fishers found a largely negative view of marine litter which they associated with; fouling, damage to gear and propeller entanglement which affected their catch as well as posed a safety hazard (Nash, 1992; Wallace, 1990). Fishers may also be impacted economically through microplastic impacts on fish species. Intestinal blockage, physical damage, histopathological alterations in the intestines, change in behaviour, change in lipid metabolism and transfer to the liver are some observed effects of microplastics ingestion (Jovanović, 2017). The Irish Seafood industry employs nearly 16,000 people either directly or indirectly, has 1993 registered vessels, 296 registered aquaculture sites and is worth approximately 1.3 billion euro (BIM, 2022). Furthermore, there exists dozens of angling societies and clubs across the country enabling people to take part in a leisurely outdoor activity which rely on stable fish populations (Angling Ireland, 2022).

The ROI has taken steps to limit the introduction of plastic waste to the environment and also to encourage its removal. These actions include; the introduction of legislation relating to banning microbeads, introducing levies on plastic bags in shops and the establishment of Bord Iascaigh Mhara's Fishing for Litter scheme in 2015 which is part of a European wide initiative. The ban on the sale of certain single use products (e.g., straws, stirrers, cutlery) came into force into July 2021 and soft plastics have been have been reintroduced to the ROI's recycling list since September 2021. Over the last few years legislation regarding proper plastic waste management have been implemented (e.g., Delegated Regulation (EU) 2020/2174 . At national level, the waste action plan of the ROI (2020-2025) intends for packaging on the Irish market to be reusable or recyclable by 2030 and introduce a deposit and return scheme. As the first country in the EU (following the United Kingdoms departure) to ban microbeads under the *Microbeads (Prohibition) Act 2019 (S.I. No. 36 of 2020), art. 2* which came into effect on the 20th of February 2020, the ROI was at the forefront of action against microplastics. However, this ban does not include products that protect from UV light such as sunscreen which are still permitted to contain microbeads. Microbead-containing sunblocks may be washed from people swimming in the marine environment, directly entering coastal ecosystems. Furthermore, no such action has been taken against products which can shed microplastic fibres such as polyester clothing which are regarded as one of the main microplastic pollutants in the environment (Herzke *et al*. 2021; Acharya *et al*. 2021; Cole, 2016; Gago *et al*. 2018).

One success story is the aforementioned Fishing for Litter initiative in ROI (BIM, 2022) which was established in 2015 with the support of the European Maritime and Fisheries Fund. The scheme encourages commercial fishing ships to return any waste material to shore that they might pull in during their fishing activities rather than discarding back overboard as might have been the case in the past. By the end of 2019, 95% of trawlers (244 boats/vessels) operating from the ROI's 12 main fishing ports had joined the programme. Large hardwearing bags are given to trawlers for the collection of waste they collect from fishing and when full are moved into a designated skip by harbour staff. To date 409 tonnes of marine litter have been retrieved via this initiative (BIM, 2023).

In order to understand the pathways of plastic waste and microplastics as breakdown products entering the environment and reduce those quantities it is crucial to understand the role that humans play in the process. The human dimension in plastic pollution and microplastics is threefold. Namely; plastics are entirely anthropogenic in nature, microplastics can have a negative impact on humans (ingestion of microplastics in diet, debris littering areas of natural beauty etc.) and humans can help to address the problem (Pahl and Wyles, 2017).

While studies have been carried out in the past on the knowledge levels, attitudes and awareness of the public towards topics such as marine litter, marine threats, plastic pollution and microplastics research gaps still exist. Very little work has been carried out on understanding these aspects in a group that may be directly exposed to issues around marine litter and in particular microplastic pollution, i.e., fishers. For example; stakeholder perceptions of marine plastic waste management in the United Kingdom was examined across 22 different groups including; marine advisors in government agencies, marine biologists, maritime researchers and enquiries officer of a marine NGO (McNicholas and Cotton, 2019), however, fishers were omitted from this study. Limited data is available on fishers and their perceptions with regard to topics surrounding plastics with one study examining fishmongers, commercial fishers and recreational fishers in a fishery in South Australia showing that all three groups misperceived plastic pollution as less of an issue locally than internationally (Wootton *et al*. 2021).

This knowledge gap needs to be addressed as the fishing industry is both a starting point of plastic entering the marine environment (lost fishing gear) and likely an industry that may be affected by microplastic pollution in the future as microplastics have been found in fishmeal for aquaculture and commercial fish species already (Thiele *et al*. 2021; Di Giacinto *et al*. 2023). Abandoned, lost, or otherwise discarded fishing gear (ALDFG) containing plastics accounts for another 27% of marine litter items found on European beaches (MARE - European Commission, 2018). It is unclear how much ALDFG enters the marine environment every year. The figure of 640,000 tonnes listed by Food and Agriculture Organisation of the United Nations is based on a 1975 estimate and fails to account for the rapid expansion and modernisation of many fishing fleets across the world (Richardson *et al*. 2021). A median of 48.4 kt of fishing gear was lost during fishing that amounted to 74% of marine capture globally in 2018 (Kuczenski *et al*. 2021). The model did not include fishing gear that is abandoned or discarded intentionally. Approximately 18% of marine plastic debris in the ocean is attributed to the fishing industry (Andrady, 2011). Ghost gear is estimated to account for approximately 10% of plastic litter in oceans however they form the majority of macroplastics (>20cm) by weight found floating at the surface (>70% by weight) (Eriken *et al*. 2014). This larger fishing gear can break down to form thousands if not millions of microplastics both on shorelines and to lesser extent at the surface of the sea. While as much as 80% of plastic that ends up in the marine environment stems from terrestrial sources it is fishers and marine-based groups that must deal with the consequences of this pollution (Andrady, 2011). Post-mortems conducted on dead dolphins found washed ashore on the West Coast of the ROI attributed their deaths to entanglement with fishing gear (IWDG, 2015). Given the dangers posed by ALDFG it is important therefore to understand the perceptions and attitudes that fishers have towards topics such as recycling, marine litter, microplastics and plastic. This in turn can lead to widely acceptable solutions to help mitigate litter inputs from fishing activities into the both freshwater and marine environments.

Fishers currently operate in a world with multiple stakeholders and the way society perceives environmental consequences of fishing is changing (OSPAR, 2018). Given the fact that fishing gear is an important source of litter entering aquatic environments and therefore microplastics generation coupled with microplastics presence being noted in many species of fish indicate that fishers are an important group to assess with regards to their attitudes, awareness and behaviour towards topics such as plastics, recycling and microplastics.

Understanding the views of Irish fishers on plastic pollution mitigation methods i.e., recycling as well as their behaviour towards litter they encounter during their fishing activities will help to develop effective strategies to hopefully reduce the leakage of fishing plastics into the aquatic environment. As noted by Wootton *et al*. (2021) understanding the perceptions of this group of important stakeholders have on plastic pollution will hopefully generate solutions that can effectively address this environmental issue that are more easily accepted by multiple stakeholders. The importance of studying the perceptions and attitudes to plastic and microplastic pollution cannot be understated as they are largely unexplored and deserving of more attention as it is human behaviour that leads to its occurrence in natural environments (Deng *et al*. 2020). With this in mind, the views of members of the Irish fishing community were examined in order to understand their perceptions, awareness and behaviour around plastics and associated topics. It is hoped that the findings of this work will aid in effective policy development and help to mitigate plastic pollution entering the aquatic environment of Ireland.

6.3 Research Aims

As far as the authors are aware this is the first study of its type in the ROI which examines the awareness and knowledge levels, attitudes and behaviours of stakeholders involved in the Irish fishing community addressing the following research questions:

1. How knowledgeable and aware are Irish fishers about plastic and the impact of plastic waste on the environment?
2. What are the attitudes of fishers toward plastic use in fishing and recycling?
3. What behaviours do Irish fishers exhibit with regard to plastic waste?

6.4 Methodology

A mixed method survey was designed in order to answer the overlying research questions. Given that a deep understanding of fishers' attitudes and concerns was desired, the mixed method approach of a combination of not only qualitative but also quantitative data was selected as the best way to achieve this. Mixed method surveying allows the combining of the two aforementioned types of data which can complement each other and enables real experiences in qualitative responses to be matched with quantitative data which helps to validate the results of the survey (Cresswell and Cresswell, 2018). The coupling of two different data gathering methods in this survey enable the real-world observations of fishers from the aquatic environment to be quantified and their interpretivism around subjective experiences and their own understanding of environmental topics such as recycling and microplastics to be expanded on (Wasti *et al*. 2022).

6.5 Survey development and participants

The survey was initially developed and refined by consultation within the authors' research group. The survey was then formally piloted with a random sample of fishers in September of 2020 trialling physical copies of the survey and minor modifications were made following this. Each respondent was asked 26 questions which included demographic information followed by questions addressing the awareness, knowledge, attitudes and behaviour of the respondents in relation to plastic pollution, plastic use in fishing and management of plastic waste. This study was conducted among individuals involved with fishing (both commercially and recreationally), angling, fish processing and aquaculture in the Republic of Ireland, during the period from May to November 2021. Those involved in scientific research relating to fisheries were also eligible to participate. Participants that did not match these criteria were excluded.

6.6 Sample Size

The revised survey was administered online via google forms from May 2021 to November 2021 and distributed by email and social media platforms. To specifically target stakeholders involved in the Irish Fishing community the survey was published on the Inland Fisheries Ireland angling website (www://fishinginireland.info/2021/fishing-updates/contribute-to-research-into-plastic-pollution-in-our-waterways/) and emailed to subscribers of the "Irish angling update newsletter".

6.7 Ethics and consent

The research underwent a full, thorough, internal, institutional ethical review in Dundalk Institute of Technology and was conducted in accordance with Standard Operating Procedures (SOPs), adapted from the model SOPs developed by the Association of Research Ethics Committees (Association of Research Ethics Committees (AREC) 2013). No information was withheld and the minimal risks were clearly explained through informed consent. To ensure confidentiality, anonymity was assured at all times. All data were stored safely and securely in accordance with institutional policies. The ethics committee at Dundalk Institute of Technology approved the survey.

6.8 Data Analysis

Data were cleaned and analysed statistically using the Minitab statistical software package (version Minitab® 21.1.1 (64-bit)). Descriptive analysis was conducted, and data were reported as percentage and frequency. Answers to the open ended; 'Please give your understanding of the term microplastic?' were graded and given a score out of six. Scores of '0-2' indicated no or little knowledge of the topic, 3-4 indicated an adequate understanding of the topic and scores 5-6 were given to answers which displayed a high level of understanding of the topic. The knowledge scores data were tested for normality using the Anderson-Darling test, which revealed it was non-parametric. Following this median knowledge score of microplastics vs the age of participants was examined for significant differences using Kruskall-Wallis test (significant if p value less than 0.05) using Minitab statistical analysis. Content analysis was carried out on open-ended responses and these were analysed in order to identify trends and themes and interpret the data effectively. Graphs were constructed using Microsoft Excel 2016.

Data regarding participant attitudes towards plastics and recycling was also collected. The terms were analysed through inductive content analysis (Vaismoradi *et al*. 2013; Dilkes-Hoffman, 2019; King *et al*. 2023). Word themes/stems or synonyms were identified and then word frequency was determined for each identified word/phrase. A word cloud was constructed from all words reported by two or more participants using the freely available online program 'wordart' (http://www.wordart.com/). Font size was used to depict word frequency and font colour to represent negative, positive and neutral connotations. Red was selected to portray negative words/phrases, green for positive words/phrases and blue for words/phrases deemed to be neutral / ambiguous (Vrain and Lovett, 2020). Words were classed as negative, positive or neutral / ambiguous through individual assessment and post-assessment comparison between the authors (Dilkes-Hoffman *et al*. 2019).

6.9 Results & Discussion

6.9.1 Demographic Information

In total 73 responses were received to the survey (70 males and 3 females), after data cleaning, 72 responses remained for final analysis (70 males and 2 females). The demographic and activity breakdown of respondents are presented in Figure 6-2 below. Although there was a lower amount of respondents than hoped for, the number of responses received is not dissimilar to that obtained when Wyles *et al*. (2019) surveyed the fishing community in the UK (n=97) (all male). This small number of respondents can be due to the difficulty of reaching this specialized community (e.g. fishers being at sea sometimes 6–10 days at a time) (Wyles *et al*. 2019). From the authors' own experience communicating with this particular cohort can be difficult. Many fishers, especially older fishers have very little or no online presence (lacking social media or email addresses), however, an online only version was used following the pilot study results as when completing physical copies of the survey respondents were able to skip questions which led to patchy data collection with incomplete surveys being returned. The online survey required every question to be completed and so they had a 100% completion rate. The age profile of respondents skewed towards the older age categories (35-44≤), this is in contrast to the results of a meta-analysis of surveys which found that generally age has a negative relationship with response rates (Wu *et al*. 2022). Amongst respondents, 63% had been involved in fishing activities for more than 20 years which may bias data as not being representative of the Irish fishing industry as a whole (i.e., more traditional views and methods to handling litter may prevail). Considering that in 2017 women made up just 7% of those employed in the Irish seafood industry it was not surprising that 97% of respondents were male (Fisheries and Aquaculture, 2017). The majority of respondents (75%) selected angling as their primary fishing activity and 15% selected commercial fishing (Fig. 6-2).

Figure 6-2: Chosen activities, experience levels, and age categories of respondents.

Just over half of respondents selected their location as counties with coastline (53%) and with 66% reporting that they spent a considerable amount of time in the natural environment when partaking in their selected activity (once-a-week or more frequently). Additionally, 14% of responses were categorised as a non-specific

Irish location and included terms such as 'Eire', 'Southern Ireland', 'South west', or 'all over the country' for example. These non-specific Irish locations likely refer to vessels that leave Irish coasts but travel far out to sea for fishing purposes.

6.9.2 Irish fishers have a high level of awareness on plastic pollution

A majority of those surveyed (68%) stated that they had noticed an increase in the use of plastics in their selected activity, 25% stated they had not while 7% said that they did not know. This may be due to the fact that over the past few decades there has been an increased reliance on plastic in fishing activities in the form of nets, lines, ropes etc., with the lightness, durability, buoyancy and low cost making it ideal (Watson *et al*. 2006; Andrady, 2011, Wootton *et al*. 2021). While overall there was a noted increase in the use of plastics in fishing activities amongst survey respondents this was not always the case when examined in relation to the experience level of respondents. The majority of those involved in their fishing activity for between 11-15 years stated they had not noticed an increase in plastic use in their activity, while half in the experience category of 6-10 years said they also had not. Conversely 75% of those in the ‘20+ years’ experience category stated that they had noticed an increase while nearly 80% in the least experienced category of '0-5 years' also noticed an increase despite their relatively short time spent in their selected activity, this was however a smaller group than the aforementioned one (Fig. 6-3).

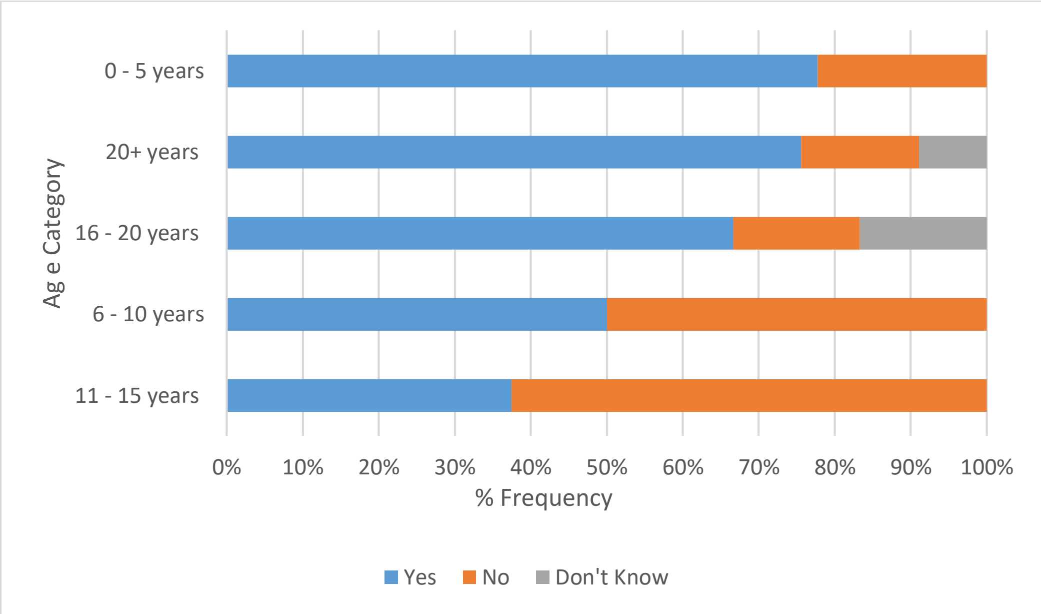

Figure 6-3: Experience of respondents to survey and if they had noticed an increase in plastic use in their selected activity over time.

Waste material seems to be ubiquitous in environments where fishing occurs in ROI. When fishers were asked 'when partaking in your selected fishing activity how often would you notice the presence of items that may be classed as waste material (e.g. plastic bags, water bottles, discarded fishing lines, etc.)?' 43% and 31% of respondents respectively stated 'Every time' or 'often'. A smaller portion consisting of 22% of those surveyed said they noticed waste material only 'occasionally' while the final 4% stated they 'rarely' did. Given than no respondents stated they never saw litter this would mean that 100% of those surveyed noticed litter when taking part in their fishing activity highlighting the ubiquity of litter in aquatic environments (Fig. 6-4). This is in line with other results that have examined similar topics. For example, the MARLISCO survey (3748 respondents from 16 European countries) found that most people reported that they noticed marine litter on most or every visit to the coast and that they said this situation was deteriorating (MARine Litter in Europe Seas: Social AwarenesS and CO-Responsibility, 2015). While 81% of members of the public declared they witnessed plastic pollution on a ‘daily’ or ‘weekly’ basis in UK-based focus groups (Henderson and Green, 2020). In the current study, participants were also asked to numerate the amount of waste they normally notice when conducting their normal fishing related activity; 'when partaking in your selected fishing activity how many items would you normally notice that may be classed as waste material (e.g. plastic bags, water bottles, discarded fishing lines, etc.)?'. The largest amount of responses fell into the '10+ items’ at 35%. This was followed by; '1-3 items' at 29%, '4-6 items' at 25% and '7 - 10 items' at 10% of total responses. The category with the lowest amount of responses was '0 items' which had just 1 response (Fig 6-4).

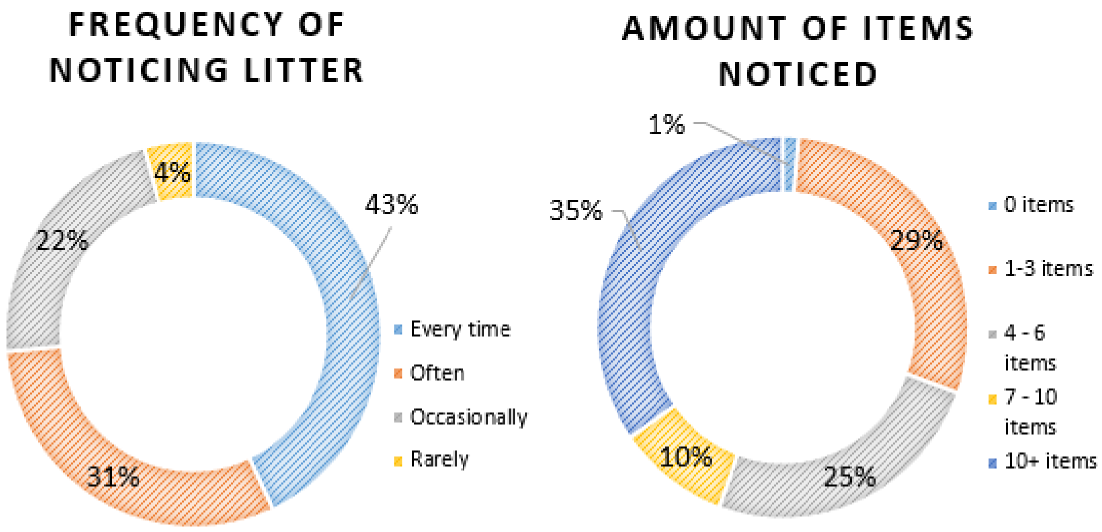

Figure 6-4: Fishers interactions with litter in aquatic environments.

Fishers were found to have a high level of awareness on the topic of microplastics (Fig. 6-5). Eighty five percent (85%) of respondents answered they had heard of the term microplastics previously with 11% stating they had not and 4% stated they 'don't know' with a further breakdown shown in Figure 6-5. This is much higher than reported by focus groups on the general public in the UK where very few of the participants had heard of the term (Henderson and Green, 2020) and from UK members of the public where 68% of survey respondents did not know what microbeads were (Greenpeace, 2016). This may underline the fact fishers have a vested interest in this environmental pollutant and are therefore more aware.

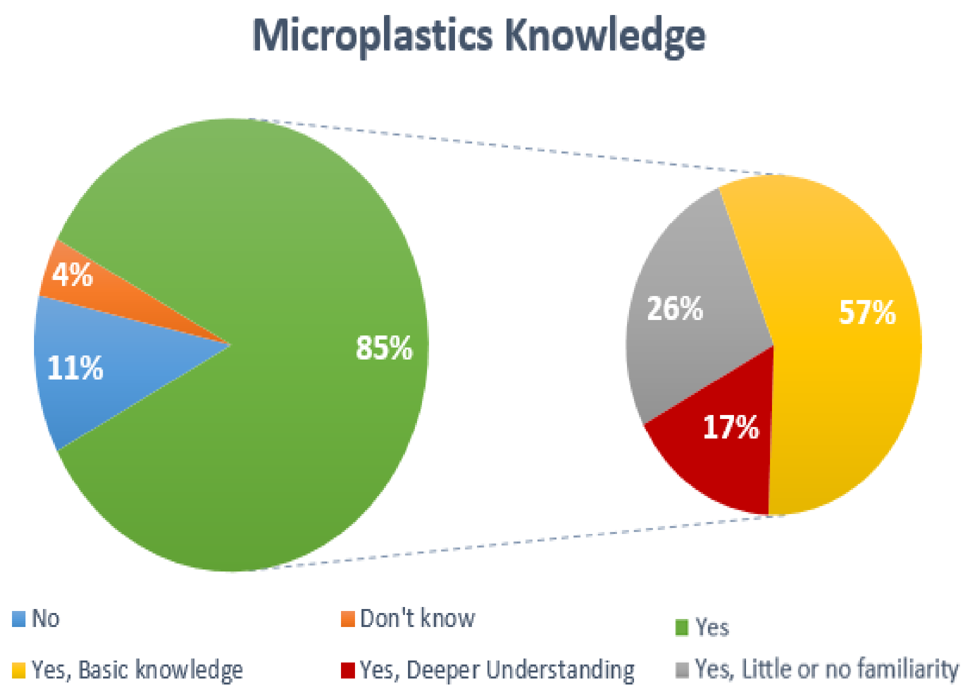

Figure 6-5: The breakdown of respondents that had heard of the term microplastics before and their subsequent knowledge levels.

Some examples of highest-scoring responses included: '*Break-down of plastic particles suspended in waters at a microscopic level*' or '*plastic which has been broken up into small pieces so it is no longer visible and very difficult to take out of the water*'. Examples of lowest scoring answers besides the null responses which earned 0 as a score include: '*microplastics are used in products such as face washes*' and '*in shampoo, etc.*'. Of survey respondents that gave a description to what they thought microplastics were; 33% mentioned a source (breakdown / designed) and included phrases such as; '*by-product from manufacturing*', '*large plastic breaking down*' and '*found in shampoo*'. Additionally, 35% of responses included a 'location / area of effect' of microplastics and mentioned terms such as; '*small tiny plastic fragments, that fish usually eat*', '*small plastics that plankton and aquatic life will feed on*' and '*microplastics are broken down particles of larger plastic pieces which can enter the food chain by being eaten by organisms or absorbed by organisms*'. Interestingly one respondent stated '*Microscopic plastics in water which impact fish and aquatic life. Most I've seen has been related to the ocean, not freshwater*', indicating that they were more knowledgeable on the topic with regards to the marine environment than freshwater. This knowledge was noted in those surveyed as a whole and was further evident in their responses to awareness of plastic pollution and microplastics in various environments (Fig. 6-6). Notably, microfibres were not mentioned in the responses of any who provided responses to this question potentially indicating that fishers are unaware that fishing gear and clothing can create microplastics.

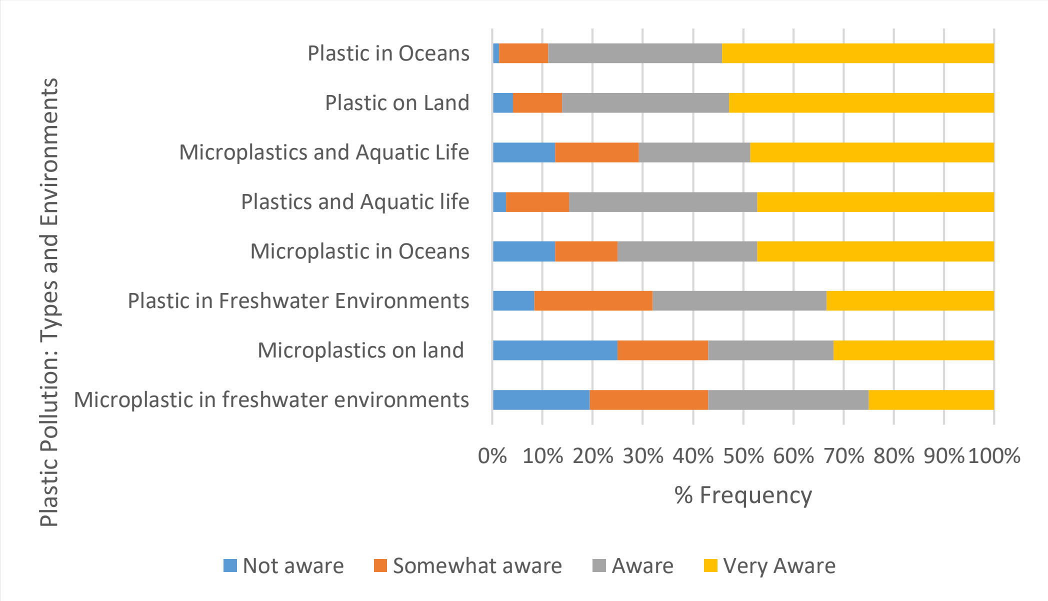

Figure 6-6: Respondent's awareness of plastic pollution in various environments.

Following this, the total scores of microplastic understanding and the mean score per respondent were tallied for each age group (Fig. 6-7). The age category group; '55-64' had the highest mean score for microplastic understanding amongst those surveyed at 3.57. The lowest mean score was seen in the oldest age category surveyed; the age category '65+' which had a mean score of just 2.8. The remaining 4 age categories (18-24, 25-34, 35-44, 45-54) all returned mean scores of microplastic knowledge of; 3, 2.63, 3.06 and 3.26 respectively. As data was deemed to be not normally distributed via the Anderson-Darling test for normality Kruskall-Wallis analysis was carried out. This analysis found that there was no statistical difference between the median knowledge values of each category ($P = 0.927$). The mean score for all age categories was 3.15 ± 1.66 which falls into the bracket of having basic knowledge of microplastics while the median score was 4 which falls into the same score bracket.

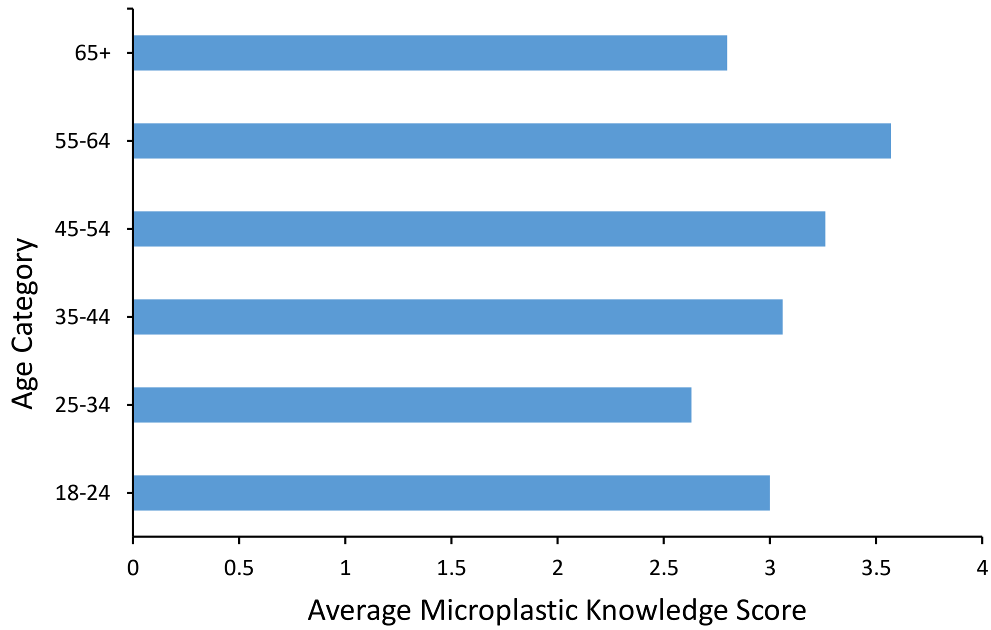

Figure 6-7: Mean microplastic knowledge score for age categories.

The understanding and awareness of the impact of plastic pollution and microplastics on various environments and aquatic life was measured using a Likert scale. Overall levels of awareness by fishers on all topics was generally quite high (Fig. 6-7). Percentage responses in 'aware' and 'very aware' categories ranged from 57% for the topics; 'Microplastics on land' and 'microplastics in freshwater environments' to 89% for the topic: 'Plastic in the ocean'. This high level of awareness may be due to the media coverage of topics such as the great pacific garbage patch and footage of cetaceans interacting with plastic items in recent years. When assessing the same environment (oceans) on the topic of microplastic pollution awareness was still quite high with 75% of participants 'aware' or 'very aware'. This difference in awareness levels may be due to the fact that macroplastic or plastic pollution is a more tangible thing that can be witnessed with the naked eye in natural environments while in general microplastics will not be visible to participants in fishing-based activities without searching for them or using specialised lab equipment. Despite these differences microplastic awareness was still quite high especially for the topic; 'Microplastics in oceans'. Recent emphasis in the media on research on these topics and television shows (e.g., Food Unwrapped: the plastic in our food, 2017, the Irish state broadcaster, RTÉ sharing news items on microplastics e.g. *'How wet wipes and sanitary products are* *causing marine pollution'* (Morrison, 2020)) discussing these topics may be creating this, perhaps unexpected, awareness amongst the cohort surveyed. This indicates an awareness among respondents about the negative effects of plastic on the environment, which is aligned with other studies (Filho *et al*. 2021; Kershaw *et al*. 2011; Otsyina *et al*. 2018). This is reflected in the wordcloud (Fig. 6-10) generated where the majority (53%) of phrases associated with plastics were deemed negative. The potential impact of media, although unquantified in this study, cannot be understated as media help to simplify complex scientific issues and topics and present them in a 'storyline' format in which audiences can engage with moral responsibility and interpretation (Entman, 1993; Gamson and Modigliani, 1989). Furthermore, media outlets tend to sensationalise scientific findings in order to make a more exciting or attractive story and for example; 'microplastics in the human body' makes an attention-grabbing, scary or 'clickbait' headline (Dempster *et al*. 2022; Chakraborty *et al*. 2017; Völker *et al*. 2020). Additionally, the nature of global news and social media enables the blame and guilt associated with plastic use to be shifted from individuals to other groups (Wootton *et al*. 2021).

Overall, microplastics on land followed by microplastics in freshwater environments were the topics with the highest selection of 'not aware' at 25% and 19% respectively. The selection of not aware on the topic of microplastics on land was twice as high when compared to the selection of not aware on the topics of microplastics in oceans and the topic of microplastic pollution interacting with marine or riverine life (fish, insects, birds etc.) and links in with the understanding of the term microplastics of several respondents. While at first glance it may appear that fishers seem to be less aware of impacts or topics that they view as unrelated to the area they carry out their primary fishing activity in (i.e., terrestrial environment and microplastic impacts). The same was noted in Irish farmers who felt they knew more about microplastics and plastic pollution in aquatic environments than on land (King *et al*. 2023).

Respondents were asked to rank several different topics on how serious they view the threat of plastic pollution towards them (Fig. 6-8). Overall, the perceived threat of plastic pollution was high; this ranged from 68% (serious or very serious) for the threat to 'humans' or 'land' to a high of 86% for oceans. This ranking of plastic pollution threat to the oceans as the topic with the highest amount of responses of 'serious' or 'very serious' is in keeping with the findings of Dilkes-Hoffman *et al*. (2019) that 69% of members of the public rated plastic in the ocean as very serious. The topic with the highest percentage (57%) of responses in the 'very serious' category was 'freshwater and marine wildlife' and obtained 81% of responses in the combined 'very serious' and 'serious' categories. This likely reflects the fact that Irish fishers' livelihoods and recreational activities depend on the wellbeing of fish stocks now and in the future. The responses of this cohort of Irish fishers is in keeping with the views of the Irish public in general, as 85% were 'extremely concerned' or 'very concerned' about the impacts of plastic on ocean health and marine life (EPA – Plastics report, 2022). The threat of plastic pollution to humans while still perceived as high albeit reduced compared to the aforementioned topics. Receiving 68% of responses in this study in the 'very serious' and 'serious' categories was also similar to the responses of the EPA study of the general public on the 'potential human health impacts' which received 74% of responses in the 'extremely concerned' or 'very concerned' categories.

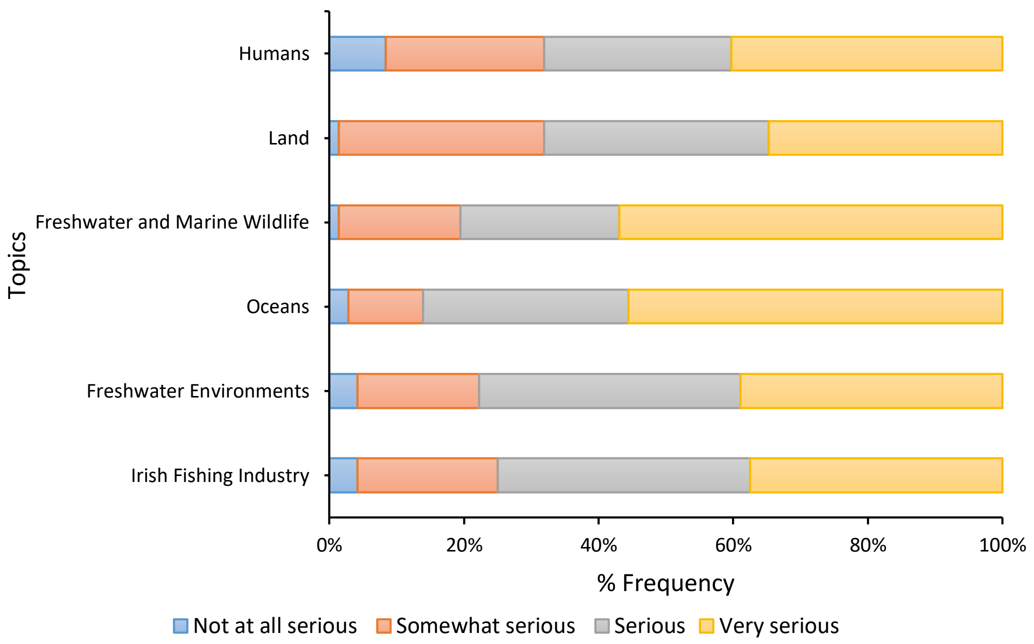

Figure 6-8: Respondents ranking of threat of plastic pollution towards different topics.

6.9.3 Irish fishers view plastic as convenient and cheap but damaging to the environment

The next part of the survey sought to understand fishers' attitudes towards plastic pollution and their general views of plastics. Responses to the following question; 'where do you think plastic pollution in rivers / the ocean is coming from?' were categorised into the following; domestic, fishing, agricultural, littering, other and anthropogenic but non-specific (Fig. 6-9) and sought to understand who Irish fishers' view as being responsible for plastic pollution of aquatic environments. Anthropogenic but non-specific was the most commonly seen response to sources of plastic pollution in rivers/oceans with 29% of responses falling in this category. Littering was the next most common response (21%) followed by; other (14%), fishing (13%), domestic (12%), and industry (6%). The category with the lowest percentage of responses was agriculture (5%). 'People' was the most common answer, mentioned 11 times in responses while 'humans' were mentioned 5 times. Anthropogenic sources i.e., 'humans' or 'people' were the most common answers. This would indicate that the majority of those surveyed understand that plastic pollution is a human problem they seem to place the blame at the foot of individuals i.e., 'careless leisure seekers' or 'lazy people' instead of larger sectors such as industry and agriculture which were the two least commonly seen responses. This would indicate that in the eyes of this cohort from the Irish fishing community a disconnect exists between the polluting of waterbodies and larger scale activities. Although blame for pollution of rivers/oceans was placed at the foot of individuals no respondents said that they themselves caused littering and instead described those that did cause littering as 'lazy' or those lacking in education; 'lack of education to bin properly'. This is in line with previous studies (e.g., Campbell *et al*. 2014; Santos *et al*. 2005; Slavin *et al*. 2012) that found despite generally claiming not to be responsible for littering themselves members of the public identified beach users as the main source of marine litter. This blame allocation for littering may stem from traditional schemes in the ROI where the emphasis for litter management and recycling has been placed at the foot of the public rather than at that of corporations. An example of which was the introduction of the 15-cent levy on plastic bags at the point of sale for consumers in the ROI (Marlisco, 2002).

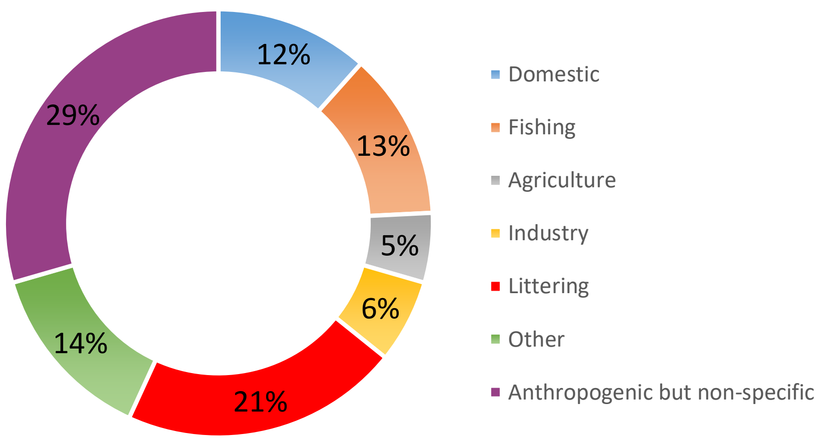

Figure 6-9: Perceived plastic pollution sources of aquatic environments.

Given that members of the Irish fishing community surveyed recognised that fishing activities create plastic pollution in aquatic environments they may be open to novel methods to reduce this occurrence. An example on how to reduce the amount of ALDFG generated is via radio frequency identification (RFID) tags which can be installed on fishing gears such as buoys or highflyers of gillnets and longlines as gear marks. The only RFID technology test for fishing gear position marking was a pilot study by Irish Fisheries Board (BIM, 2007) and found that off-the-shelf commercial equipment had a range of approximately 240m and may be useful for the recovery of lost gear where the general location is known (He and Suuronen, 2018). Furthermore, significant interest has been generated in ropeless trap / pot fishing due to an increase in humpback whale entanglements and the 2017 North Atlantic right whale unusual mortality event (Myers *et al*. 2019). A switch to more high-tech fishing gear and tags may lead to a reduction in ALDFG, which could be driven by the introduction of subsidies by government to fund this change as evidenced by the ‘Ghost Gear Program’ in Canada (Fisheries and Oceans (DFO) Canada, 2021). The Ghost Gear Program funded by the Canadian government has supported 49 projects under four program pillars: 1) ghost gear retrieval; 2) responsible disposal; 3) uptake and piloting of technology to prevent gear loss; and, 4) international leadership (Fisheries and Oceans (DFO) Canada, 2021).

Another open-ended question put to respondents; 'please list below two words/phrases you associate with plastics (either positive or negative)'. For ease of interpretation the results are presented as a word cloud (Fig. 6-10). All of the responses were categorised as either positive, negative or neutral. Terms with positive connotations made up 37% of the total comments with cost 'cheap' (n = 10) and convenience (n = 10) being the most common responses. The only other terms that were mentioned more than once were; 'recycle', 'durable' and 'essential' with the latter two terms relating to the functionality of plastics and durability a recurring theme from fishers selection of using specific fishing plastics. The majority of comments, however, were deemed to be negative (53%). General environmental concern (n = 39) ('damaging to the environment', 'environment killer'), association with litter and pollution (n =7) ('unsightly', 'dumping') and the long-term and persistent nature of plastics ('microplastic', 'non-degradable') were the key reasons that respondents viewed plastics negatively. These results were similar to those found in a study by Dilkes-Hoffman *et al*. (2019) carried out on members of the public in which the same question was asked and led to positive answers such as; 'cost (cheap)', 'convenience' and 'usefulness' and negative responses including; 'waste/rubbish', 'pollution' and 'environment'. In this regard fishers are a reflection of members of the public studied by Dilkes-Hoffman *et al*. (2019) in that they both recognise the usefulness of plastic material but also see it as something that can have damaging effects to the environment and human interaction with it. Additionally, 22% of respondents included both a positive and negative response to this question which reflects the duality of plastics in modern life and included responses such as; 'damaging, durable', 'cheap but not cheerful' and 'carcinogenic, convenient'. Further inductive analysis was carried out on the positive and negative phrases that fishers used to describe plastics. Amongst positive terms used by respondents, the majority, 67%, were focused on the general usability or functionality of plastic, 19% on the end of life and the remaining 14% categorised as general positive terms. General negative terms made up the majority of the negative responses (46%), followed by end of life issues / concerns (25%), the ubiquity of plastic / lack of alternatives (15%) and environmental issues making up the remaining 14% of phrases. The complete list of phrases mentioned along with frequency is displayed in Table 6-1.

Figure 6-10: Wordcloud displaying phrases fishers used to describe plastics.

Table 6-1: Phrases associated with plastics.

Positive	Frequency	Negative	Frequency	Neutral	Frequency
convenient	10	litter	1	indestructible	1
durable	4	dumping	1	ubiquitous	1
good	1	unnecessary	2	packaging	1
cheap	10	unsightly	1	developing countries	1
easy cleaned	1	bad	1	mulroy Bay	1
essential	2	damaging	3	disposable	1
useful	1	not environmentally friendly	1	less plastic	1
easy	1	pollution	1	long lasting	2
handy	1	not enough alternatives	1	beach goers	1
positive	1	too prevalent	1	no bin	1
recycle	5	not needed	1		
reuse	1	ever lasting	1		
biodegradable	1	Persistent	1		
non-toxic	1	microplastic	1		
depend on it	1	non degradable	1		
perfect packaging	1	carcinogenic	1		
		single use	4		
		irresponsible	1		
		lazy	1		
		thoughtless	1		

should be banned		1
lifetime	1	
negative	3	
no alternative	1	
non recyclable	2	
everywhere	1	
disgusting	1	
terrible	1	
detrimental	1	
harmful	1	
needless	1	
permanent	1	
crap	1	
unnecessary	1	
not cheerful	1	
painful	1	
useless	1	
poison	1	
dirty	1	
environment killer	1	
rubbish	2	
pollution	2	
lifelong	1	
bad	1	
nonbiodegradable	1	
longterm problem	1	
not good	1	
serious problem	1	
damaging to environment	1	

Fishers were asked to rate their level of concern towards the amount of plastic currently used in fishing equipment with the highest proportion of respondents (33%) saying they were ‘concerned’ and only 14% were ‘not at all concerned’ in this regard (Fig. 6-11). The high levels of concern that Irish fishers feel towards the current levels of plastic use in fishing equipment coupled with the fact that the lack of alternatives for plastics was mentioned in their response to opinion on plastics it is likely that they would be open to switching to more sustainable replacement equipment as was noted in stakeholders in an Australian fishery (Wootton *et al*. 2021). However, this change could only occur if it was convenient, easy and cheap for recreational and commercial fishers and fishmongers (Wotton *et al*. 2021).

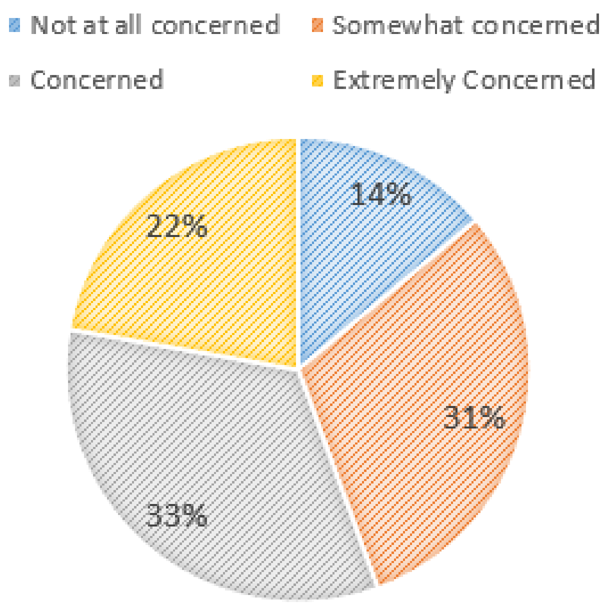

Figure 6-11: Concern levels of Irish fishers on amount of plastic used in fishing equipment.

6.9.4 Fisher's interactions with litter and fishing plastics

The behaviour of respondants towards plastic use in fishing, their recycling habits and how they deal with litter in the environment was also examined. Respondents were asked; 'when conducting your fishing-based activity how likely are you to remove waste material you notice from the natural environment?'. This was examined as comparative research on household waste management found that simply increasing awareness of environmental issues does not necessarily lead to effective practices (Skorstad and Bjørgvik, 2018). Responses fell onto a 5-point Likert scale that included the options; 'highly unlikely', 'unlikely', 'unsure', 'likely' and 'highly likely'. Respondents generally stated that they would be inclined to remove waste material they noticed from the natural environment (Fig. 6-12).

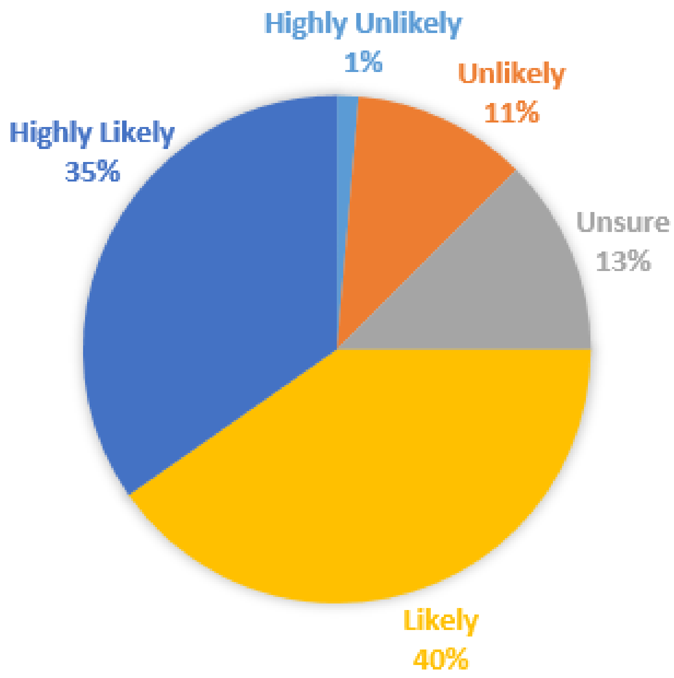

Figure 6-12: Likelihood of removing waste from aquatic environment.

Following this, the relationship was examined between the likelihood of waste removal and the fishing activity that respondents selected. Commercial fishers were the most 'likely' or 'highly likely' to remove litter they encounter while carrying out their activity (91%). This was followed by aquaculture (75%) and angling at 70% (Fig. 6-13). The fact that commercial fishers, anglers and aquaculturists were, for the most part, likely or highly likely to remove waste material indicates they may have a level of care or understanding for the need to preserve the environment they enjoy a leisure activity in or livelihood from. Fishers may potentially be associating environmental care with direct economic benefit, which is paralleled with competent fisheries management (Asche *et al*. 2018). These results would indicate that while fishers are noticing waste in the environment, they fish in both frequently and in medium-large volumes they are also likely to remove aquatic litter from the environment. This may be due to their direct experience from seeing litter frequently and understand the impacts it can have and take responsibility for its removal. A recent study on marine litter and fishers has shown that collecting waste material and storing it can be difficult on-board fishing vessels, especially smaller ones, due to issues such as; lacking capacity and lacking time and personnel for doing so (Olsen *et al*. 2020). However, interviews with fishers in the same study found that at least some fishers thought that ‘whether litter is taken ashore is much about attitudes’, as in, waste removal and collection from the environment will occur based on attitudes and is an independent factor from vessel size in general.

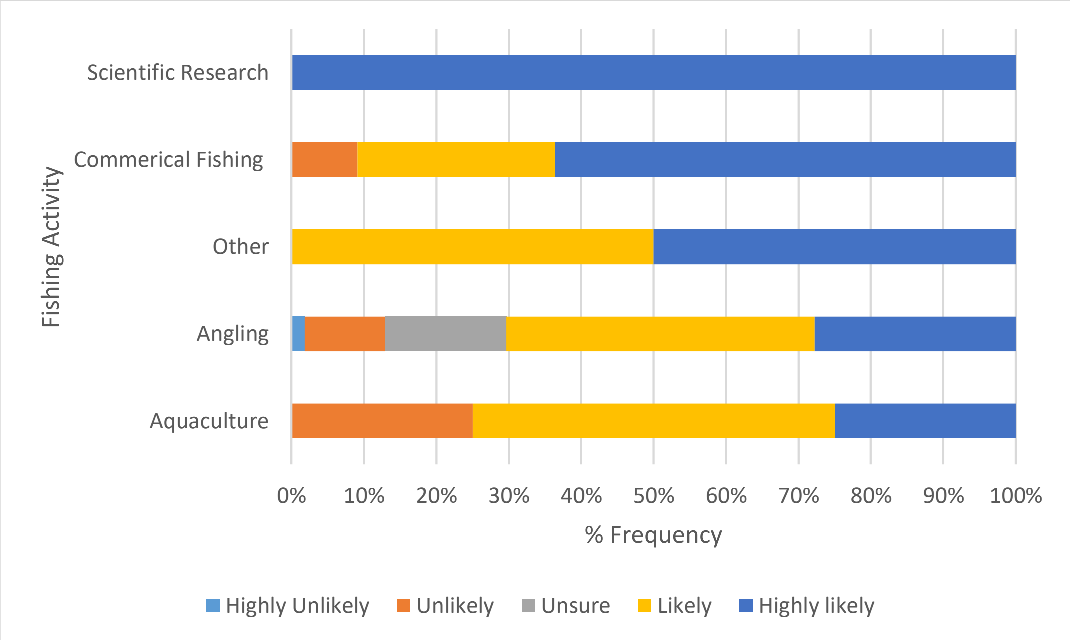

Figure 6-13: Likelihood of fishers to remove waste based on their fishing activity.

Marine plastic pollution remains a global threat with discarded fishing gear a major contributor (Kuczenski *et al*. 2021). Plastic is a major component of fishing gear including lines, netting, life jackets/waders, containers and lobster/crab pots, all of which can represent a disposal problem. In this study, respondents were asked to explain their reason for choosing certain plastic material for their selected fishing activity. Durability played a major role in the use of all plastic items, ranking first for all items save ‘Lobster / crab pots’ where cost played the greatest role in their use. Generally, recyclability and ease of disposal ranked medium-low (highest ranks of 3rd and 4th respectively out of 7 places) for reasons that fishers selected plastic items (Fig. 6-14). This would indicate that end of life concerns do not play a major part in plastic item selection by Irish fishers, with their decisions primarily based on functional or economic incentives. Members of the Irish fishing community chose plastics for use in their activities despite the fact that they are broadly aware about the impacts plastic pollution can have and see litter frequently during their activities and so awareness and knowledge is not really affecting their behaviour. However, this awareness and knowledge may mean they are open to alternative material for fishing plastics. Although not explicitly linked to this question on plastic use one individual offered up more information by selecting other and commenting; 'I would like to recycle in or disposal in a skip but the skipper directs me to dispose overboard'. This may indicate that human command may be a barrier to proper recycling habits on board Irish Sea vessels. Although recyclability did not rank in the top 2 reasons for the selection of any plastic products by Irish fishers in this study, the Irish public in general have a noted high willingness (89%) to use and buy plastic packaging and products that are made of 100% recycled plastic (EPA, 2022). The increased availability of fishing material made of recycled products could therefore lead to a more circular system for Irish fishing.

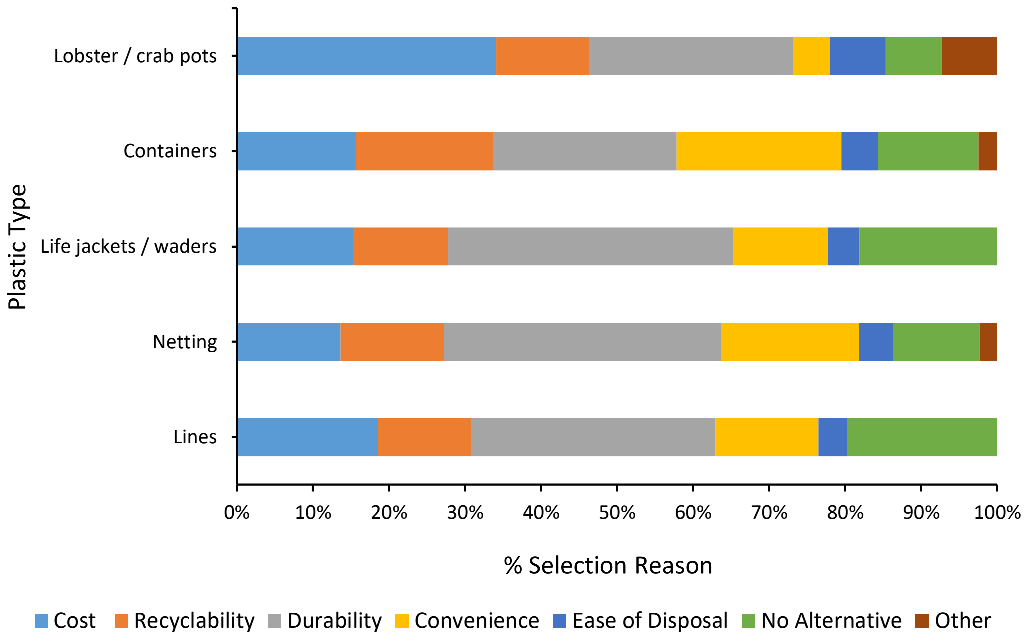

Figure 6-14: Reasons for the selection of different plastics by respondents.

Fishers were also asked to define the disposal methods they used for the aforementioned 5 groups of plastic items (Fig. 6-15). The following options were available for selection as answers; 'Pay for Disposal', 'Pay for Recycle', 'Repair / reuse', 'Domestic Waste Bin', 'Don't Know', 'I do not use these plastics' and 'Other'. For a true picture of how plastics are disposed of, the responses in the category; 'I do not use these plastics' were excluded for further analysis. Totalling the disposal methods of the 5 types of plastic items found that 72% of plastic items used were disposed of responsibly by fishers (Pay for disposal, pay for recycle, domestic waste bin), however it is unclear whether waste was segregated by fishers disposing of it. This is similar to a study carried out by Filho *et al*. (2021) on the waste management of plastics used by members of the public which found the majority of respondents (74%) segregated plastic waste and disposed of it in a responsible manner. For a true picture of how unrecycled plastics are disposed of and to facilitate a greater understanding of waste management behaviour respondents could select the response 'other' and add a comment. While 'other' was selected 25 times for various types of plastic equipment just 44% of the 25 respondents elaborated on what they did with those plastics. Several responses mentioned recycling in some form e.g. 'We put ashore to a recycle (bin) on pier head' and 'Recycle centre', two more responses mentioned burning of the plastics they use. While most troublesome of all was the response: 'Discard back into the sea, by order of the skipper' indicating deliberate littering action of the natural environment. While this response in isolation is worrying it must be noted that this is an isolated response to the survey amongst a plethora of more environmentally conscious respondents, it must also be noted the language of this response 'by order of the skipper', suggests an order a senior authority to litter the sea. In general, however, the majority of plastics used (72%) are disposed of responsibly indicating that fishers are taking responsibility for the plastics they use.

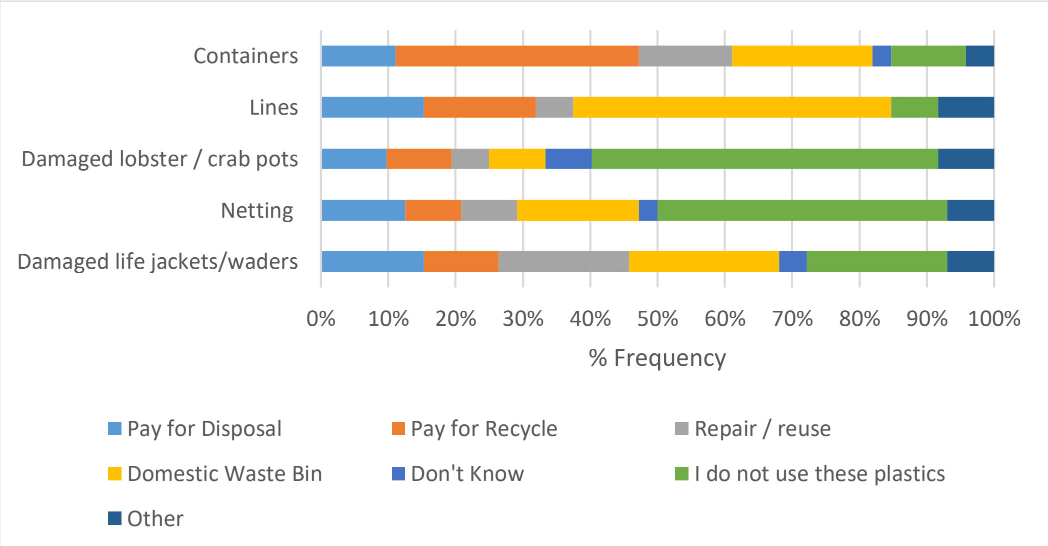

Figure 6-15: How fishers dispose of different plastic types.

Fishers play a pivotal role in aquatic litter as they can easily add to it through littering of marine or freshwater environments (e.g., through dumping at sea, or the losing of gear) as well as being in a unique role of being able to retrieve marine or aquatic litter from otherwise remote locations and habitats. It must be remembered that barriers do exist to proper recycling in the fishing community be it logistical constraints or through to human behaviour. For this reason, having proper facilities and adequate infrastructure in place to make the disposal or recycling of items that can become marine litter as cheap and convenient as possible for this group of stakeholders would be of value.

In lieu of this, respondents were asked to indicate the barriers to recycling they face when trying to dispose of common fishing plastics (Fig. 6-16). Respondents could select one of; ‘Lack of facilities’, Don’t know how to’, ‘Time consuming’, ‘Too expensive’, ‘Contamination (Not accepted)’, or ‘Other’. The options 'I do not use these plastics' and 'I recycle these plastics' were excluded to gain a true picture of the barriers faced. 'Lack of facilities' received the highest proportion of responses independent of the plastic equipment ranging from 33% to 55%. Netting was the plastic material with the highest proportion of responses in the 'too expensive' category with 17% of responses falling into this category. While 47% (n = 8) of respondents that selected ‘other’ wrote comments, two (n = 2) of these simply stated N/A. Two (n = 2) respondents stated there were no facilities to recycle fishing line. Two (n = 2) other responses indicated some form of professional waste disposal however they were unsure of the details stating; ‘I do not recycle, it’s (waste material) collected on the pier’ and ‘waste disposal company. One (n = 1) respondent stated that ‘they kept old waders to use as patches’ while finally ‘Skipper says it's too time consuming to dispose of it even in a skip at the quay. None of the boats in Wicklow town use the skip, so all litter, like drinks bottles, sweet wrappers, cling film, old ropes get thrown overboard unfortunately.’ The fact that household waste from the vessels operating out of Wicklow town may be ending up as marine litter is environmentally problematic particularly in plastic form given the myriad impacts it may have in this environment (UNEP, 2017). This statement is in direct contrast from those gathered via interview from fishers in Northern Norway (Olsen *et al*. 2020); ‘It goes without saying we take care of our own trash’. The fact that this respondent acknowledges that there is a skip available in the quay for their waste and yet their waste is still thrown overboard while out at sea is in contrast to the heavily selected category of ‘lack of facilities’ for barriers to recycling, in that facilities are available and unused. While the main barrier to recycling different plastics were physical / logistical (lack of facilities) (n = 61, 43%), the 2nd most selected barrier was a more personal option of 'don't know how to' (n = 40, 28%) and so may indicate that members of the Irish fishing community could benefit from educational courses or material detailing how to recycle the equipment they use. Interviews with fishers and fishmongers in a South Australian fishery also suggested there were a lack of appropriate disposal locations, such as rubbish bins, throughout jetties and popular fishing spots which hindered proper waste disposal (Wootton *et al*. 2021).

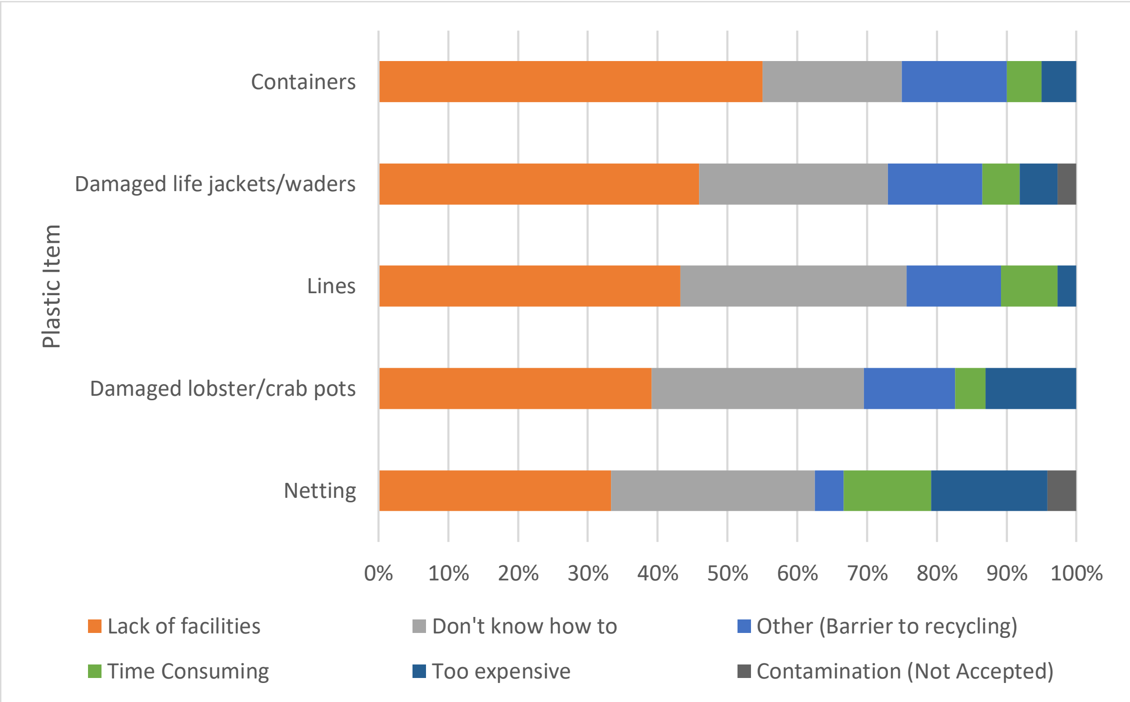

Figure 6-16: Factors stopping the recycling of plastics used in fishing activities.

In order to determine the views of recycling amongst Irish fishers, respondents were asked to list two words or phrases they associate with the topic. For ease of interpretation responses are represented in the form of a word cloud (Fig. 6-17). Responses were classed as either positive, negative or neutral. Some responses classed as neutral could also be described as non-descript in nature for example 'bin' or 'centre'. The largest portion (44%, n = 46) of terms were deemed as positive in nature with the descriptions 'good' and 'easy' recurring. The minority of terms (22%, n = 23) were classed as negative in nature. The terms 'expensive' and 'inconvenient' were commonly used while issues around recycling facilities or receptacles were also mentioned. A significant proportion (35%, n = 36) of terms were deemed as neutral ('misunderstood') or ambiguous (e.g., 'hemp-based products', 'segregation', 'plastics'). Inductive analysis of positive terms associated with recycling found that the majority were general positive comments (67%) followed by those emphasising the necessity of recycling (18%) and environmental terms made up the remaining responses (15%). With regard to the negative phrases associated with recycling the inconvenience and cost was the dominant theme noticed (52%), which was followed by the limitations of recycling / factors hindering more recycling (35%) and general negative phrases associated with recycling made up the remaining 13% of comments. The complete list of responses is displayed in table 6-2.

Figure 6-17: Wordcloud displaying phrases fishers used to describe recycling.

Table 6-2: Phrases associated with recycling.

Positive	Frequency	Negative	Frequency	Neutral	Frequency

green environment	1	inconvenient	2	limited	2
worthwhile	1	too many unrecyclable plastics	1	sometimes, benefits not clear	1
very positive	1	not enough public recycling receptacles	1	Bin	2
progressive	1	not enough	1	Centre	1
		sceptical	1	why not get rid of the plastic in the first place?	1
good	4	expensive	2	responsible	1
healthy	1	inadequate	1	misunderstood	2
important	1	costly	1	hemp based products	1
good practise	1	misguided	1	reuse better	1
achievable	1	complicated	1	plastic	1
green	2	lack of facility	1	reuse	3
environmentally conscious	1	difficult	1	not moving quick enough	1
sensible	1	Not sure which bin to use if different materials in the one unit	1	reduce waste	1
positive solution	1	not cheap	2	clean before recycling	1
good practise	1	negative	1	don't know	2
needed	2	time consuming	2	pick up and bin	1
great idea	2	recycling centre refused to take my plastics	1	segregation	1
sustainability	1	not enforced	1	proper facilities	1
easy	4	confusing	1	more facilities	1
environmental friendly	2			reduce	1
very important	1			plastics	1
positive	1			rubbish	1
Try curb its use	1			see sense	1
extremely important	1			bring your rubbish home	1
renewable	1			almost unavoidable	1
reusable	1			please recycle	1
very good	1			hard plastic	1
environment saver	1			bottle banks	1
happy	1			recycled plastic	1
friendly	1			responsibility	1
helps	1				
essential	2				
cheerful	1				
good idea	1				
necessary	1				
right thing to do	1				

6.10 Conclusion

This study provides novel insight into this understudied and unique group's attitudes. This research work should serve as a guideline for views that do exist within this niche group and is not intended to be representative of the whole Irish fishing community. This research presents a holistic first look at Irish fisher's awareness of a number of environmental issues relating to an emerging pollutant of environmental concern, i.e., microplastics. Amongst topics covered this study assessed; attitudes towards recycling and plastic, the perceived threat level of plastic pollution and the factors that hinder their recycling of plastic items they use in their fishing practises.

Fishers can be a point source of microplastics entering aquatic environments as they can be shed from discarded or lost fishing gear. Fishers are in a unique position for removing litter from remote regions and stop the production of secondary microplastics in the natural environment. Fishers can potentially be affected directly via microplastics impacting fish health or behaviour thus affecting recreational or economic activities. Furthermore, the reported presence of microplastic debris in seafood could lead to a consumer shift to different food produce in order to avoid this pollutant with fishmongers interviewed in a South Australian fishery noting that if microplastics were found in fish they sold to consumers it would wipe their business out (Wootton *et al.* 2022). While stakeholders in an Australian fishery assessed by Wootton *et al.* (2022) misperceived plastic pollution as less of an issue locally this was not the case for Irish fishers surveyed as part of this survey. Those surveyed in this current study placed the foot of the blame of plastic pollution in aquatic environments at many groups themselves included. Irish fishers were aware of microplastics and this may be due to a recent increase in news articles around this topic and their own interest in an environmental pollutant that been found in seafood around the world.

While a large amount of the ROI's fishing vessels are involved in the Fishing for Litter Initiative there is as yet no particularly innovative retrieval or preventative measures for marine litter in place in the ROI and further work is needed on this especially as it is an island nation. While the removal of litter from aquatic environments is crucial it also important to address issues such as poor waste management and unsustainable production and consumption habits which are the root causes of marine litter generation. In order for fishing to develop sustainably, fishers need the skills, knowledge and information to implement changes to more sustainable fishing gear and disposal methods. The importance of assessing this specialised group cannot be overlooked as fisher's relationship with microplastics and aquatic litter is a complicated one. Given that plastic production is expected to increase in the future and that current waste strategies are inadequate to deal with the production and consumption levels the ubiquitous presence of aquatic litter encountered by Irish fishers will continue.

Some limitations of this research are namely due to the smaller than anticipated sample size, however, as previously noted by Wyles *et al*. (2019) this cohort can be difficult to target with respect to surveying. Furthermore, this survey neglected asking fishers what could encourage them from moving away from plastic products in their recreational and commercial activities. This is an oversight on behalf of the research group; however, it can also stimulate further research questions for Irish fishing in the future and improvements in the sustainability of fishing are achieved in terms of biodegradable products and their efficacy.

The results of this study would suggest that improved and designated rather than general recycling facilities at access to fishing locations in combination with information on how to recycle fishing materials commonly used by Irish fishers could see a reduction in the amount of disused fishing equipment that may enter landfills and also the natural environment. Furthermore, fishers bemoaned a lack of alternatives to plastics available for them and were aware of the environmental issues associated with using plastics thus indicating an openness to replacement with more sustainable gear. Plastic was largely viewed as convenient by Irish fishers and this convenience would need to be replicated in replacement materials. Furthermore, any attempt to replace currently used and therefore potentially lost plastic fishing gear with more sustainable or biodegradable materials would have to ensure that these items are of equal or enhanced durability and quality in order to lead to a shift away from plastic fishing material. Furthermore, this new equipment should be similarly priced as fishers stated they generally selected fishing plastics due to these factors. Further engagement with this crucial and understudied stakeholder group should be emphasised in order to develop effective policies to reduce litter presence and generation in aquatic environments.

Chapter 7: Discussion

7.1 Overview

The research conducted and described in this thesis aimed at assessing microplastic contamination in Dundalk Bay and associated freshwater environment a previously unstudied environment with respect to this pollutant in combination with establishing a baseline of knowledge levels and attitudes towards plastics amongst a cohort of stakeholders in Irish fishing. The research and results presented in chapters 3 to 6 achieved the set objectives outlined at the start of this project. The overall study provides a holistic assessment of microplastic pollution for this important Special Protected Area (SPA) and Special Area of Conservation (SAC) by not only combining marine and freshwater research but also transdisciplinary research on the social aspects related to microplastics. The discussion that follows addresses the main findings from each chapter and helps to connect them. Furthermore, specific recommendations for helping to address and mitigate the release of microplastics to the environment as well as further research that could be conducted for the Dundalk Bay biome and microplastic research in general are also mentioned.

7.2 Achieving research objectives

The results presented in this thesis highlight the impact of freshwater systems on marine environments in terms of microplastic loadings and the concern that stakeholders in Irish fishing have to this relatively new pollutant. These results underpin that microplastics research should not occur in isolation and instead take a holistic approach to understand exposure levels to organisms and to understanding levels in one environmental compartment. As the majority of marine litter stems from the terrestrial environment it was imperative to assess the levels of microplastics present in the rivers that flow into the marine environment of Dundalk Bay before examining species that reside there.

The cyclic nature and difficulty with addressing the issue of microplastics is highlighted through the connections between the research chapters evidenced in this thesis. On the social side, stakeholders in Irish fishing were concerned by the threat posed by this pollutant to a varierty of environments and biota with concern for the ocean most emphasised. The findings from research objectives 1-3 highlight that those surveyed through research objective 4 are right to be concerned by microplastics.

As plastic production increases in the future so too shall its occurrence in the environment as waste strategies are inadequate to deal with the production and consumption levels currently seen (Walker and Fequet, 2023). The fishing industry is both a starting point of plastic entering the marine environment (Napper *et al*. 2022; Wright *et al*. 2021) and likely an industry that may be affected by microplastic pollution in the future, as microplastics have been found in fishmeal for aquaculture and commercial fish species already (Thiele *et al*. 2021; Di Giacinto *et al*. 2023). As well as through reduced revenues via public perception viewing seafood as full of plastic and a product to avoid (Wootton *et al*. 2022).

Microplastics in the form of microfibres are likely entering Dundalk Bay from myriad sources. These sources may include; surface run-off from articifical surfaces, the washing of biosolids containing microplastics into water sources, release from septic tanks, littering, fishing activites and ALDFG, WWTP effluent. Freshwater rivers entering Dundalk Bay are receiving microplastics from the terrestrial environment and transporting them to this marine environment while also storing some in sediment. Further to this, at low tide the seven freshwater rivers will continue to transport microplastics into this environment as assessed through research objective 1. In this shallow bay environment, it is likely that microplastics are accumulating faster than in other more dynamic bays as there is a lesser dilution factor reducing microplastic concentrations. The high concentrations of microplastics present in the freshwater rivers entering Dundalk Bay (research objective one) expose organisms present in the marine environment to terrestrial-sourced microplastics (research objectives two and three).

Of potential significance for stakeholders in Irish fishing is the high occurence and concentrations of microplastics in the commercially valuable, *Cereastoderma edule* and in juveniles of the fish, *Pleuronectes platessa*. Microplastics are more likely to cause harm to smaller-sized fishes and juveniles than to larger ones thus harming them in their development (Xie *et al*. 2021; Jovanović, 2017). The increased levels of microplastics found in individual cockles in autumn through research objective 2 also raises concerns of the possibility of transfer to humans due to the fishing period that coincides with this increase.

Given that similar polymers, namely polyesters and polyamide, were detected in both marine matrices examined as well as in the surface waters that flow into Dundalk Bay it is likely that the freshwater rivers constitute a significant input of microplastics to this environment. The high comparative levels of microplastics found in intertidal sediment coupled with their presence in all examined species indicate that the physical properties of Dundalk Bay are creating a hotspot of microplastic pollution accumulating this pollutant here. Further evidence of this is the fact that microfibres made up the same percentage of total microplastics recovered from both beaches in this study.

Despite the concern that this cohort expressed over plastics (realising they damage the environment) and their awareness of microplastics they still use plastic products in their activities. There is also a clear disconnect between what stakeholders in Irish fishing consider as microplastics and what presents in the environment of Dundalk Bay and its freshwaters most prominently. Those surveyed described microplastics within the context of microbeads. Microfibres were not mentioned once, e.g., "*found in cosmetics, in products such as makeup, in shampoo etc., microbeads, used in products such as face washes*". It is therefore likely that this cohort are unaware that their fishing equipment leads to microplastic release into aquatic environments even as juveniles of commercial species and marine bivalves from Dundalk Bay present high levels of microplastics thus highlighting how interconnected and difficult a problem microplastic pollution is to address.

This disconnect between what is most prevalent in aquatic environments i.e., microplastics in the form of fibres and what stakeholders in fishing surveyed in this research (who rely on a healthy aquatic environment for either recreation or their commercial interests) understand microplastics to be, underpins the role that media may have in shaping views on environmental issues as well as recent legislation banning microbeads in several products providing knowledge. For example, with regard to microplastics more media attention has been given to microbeads than to microfibres stemming from textiles which likely feeds into this gap in understanding.

7.3 Benefits of this research approach and applicability to other studies

Beginning this project river sites were selected based on the hypothesis that microplastic concentrations increased in rivers with their flow towards the Sea (Mani *et al*. 2015) and that microplastic levels would be greater at the urban study site than other more rural sites (Kunz *et al*. 2023). This site selection may have been too simplistic an approach as microplastics levels were also heightened at the rural outflow site along the River Flurry and several rivers had greater microplastic levels in their surface waters at headwater sites than outflow sites. Microplastic levels in surface water is highly dynamic and altered by flow regimes, precipitation and seasonality and proximity to point sources (D'Avignon *et al*. 2022; Horton *et al*. 2017; Browne, 2015). It can also be influenced through atmospheric deposition (Sun *et al*. 2022) so more study sites along the course of the rivers may have been a better choice to fully understand the dynamics of microplastic transport and accumulation along their courses. However, this study is intended to provide a baseline for microplastics in the freshwater environment entering Dundalk Bay and would be more applicable for an in-depth study on one river body than numerous rivers which was the subject of this thesis.

The methods used to isolate microplastics in this study were selected based on efficacy and replicability for citizen scientist projects, particularly in removing microplastics from sediment samples. For comparability of the data from this study to others 10% KOH and 30% H_2O_2 were used to digest biota samples for analysis of microplastic contamination. Tsangaris *et al*. (2021) conducted an inter-laboratory comparison of both methods and found both were effective reagents with 10% KOH being optimal. However, 30% H_2O_2 was used for the digestion of *G. duebeni* samples and water surface samples as KOH is not as efficient in degrading material from the freshwater environment (Duan *et al*. 2020). While standardisation is desirable for microplastics research in order to effectively compare studies limits of methods must be appreciated also. The choice of 10% KOH for chapter 4 and 5 of this thesis reflects the wider literature on isolating microplastics from marine species which commonly use this chemical while the use of H_2O_2 is commonly used in examining microplastics in samples pertaining to the freshwater environment. Unfortunately it is difficult if not impossible to obtain equivalence between different methods given the wide diversity of steps which can be changed (e.g., temperature, time, sample collection tool, chemical, control methods etc). For the isolation of microplastics from riverine and marine sediments, castor oil and potassium carbonate were used respectively. Both of these have displayed good recovery rates from these matrices previously (Mani *et al* 2019; Gohla *et al*. 2021) and when tested with a variety of polymers in this study. Additionally, they are non-toxic, cheap and environmentally friendly compared to other density salts such as NaI or $ZnCl_2$ (Gohla *et al*. 2021) which are regularly used in microplastics isolation from sediment. Long-term and small-scale microplastic monitoring on a local scale may be achievable through the use of citizen scientists which have been engaged in microplastic studies previously (Clark *et al*. 2023; Jones *et al*. 2022). Furthermore, these chemicals can be safely used in schools for demonstrating microplastic isolation to students and their lower comparative costs also lend themselves towards microplastic monitoring in less developed countries which may have smaller budgets for project work.

While the utmost care was taken when processing of samples, blanks revealed microplastic contamination occurring in sample processing for all chapters pertaining to microplastic isolation (chapters; three, four, five) in this thesis. Blank processing is an important step in microplastic data reporting (Dawson *et al*. 2023; Hermsen *et al*. 2018). In this study various methods were used to assess the contamination of blanks with microplastics for each chapter. The correction of results is complicated by the heterogenous suite of particles that fall under the label of 'microplastics' varying in size, shape, colour and polymer. Furthermore, secondary microplastic formation occurring in the environment or due to the separation process selected further add to a diversity of appearance (Alimi *et al*. 2022; Corcoran, 2020; Naik *et al*. 2020; Dawson *et al*. 2018; Enders *et al*. 2017). While it is now commonly known that contamination of samples with microplastics when processing occurs some studies still lack any form of contamination control (e.g., Amrutha and Warrier, 2020; Kedzierski *et al*. 2020; Zhang *et al*. 2020). During the three microplastic isolation chapters the most applicable method for ensuring the validity of results was used. For chapter one blank controls highlighted that microplastics introduced via processing were negligible to volumes recovered in freshwater matrices. For biological samples in chapter two and chapter three the mean microplastics detected in cockles / species and in blank samples were compared highlighting they were significantly different. Finally, in chapter two the average percentage that contamination introduced via sampling could have accounted for when examining marine sediment was displayed with results. Further to this, marine sediments were separated in a closed system reducing the possibility of airborne microplastics impacting on results.

Contamination controls were improved throughout the course of the thesis. Furthermore, following a visit to the cleanroom in use by Dr. Ana Mendes in University of Galway contamination prevention was strengthened. Thus, contamination controls were more vigorous for the latter stages of this work i.e., for chapter five than chapter three with measures such as sticky mats located outside of the clean room and the use of long stable cotton yarn wipes for cleaning surfaces introduced. For future research the controls used in chapter three of this thesis should be considered robust and could be used in the absence of a specialised clean room that can be utilized for microplastic isolation purposes.

There was a noted preferential ingestion for coloured microplastics in this *G. duebeni* than for transparent ones. This indicates that the presence of dyes or additives facilitate the consumption of microplastics in this species that ultimately means that this species alone is not adequate as a bioindicator and may result in skewed colour profiles that is not representative of the microplastics in the surrounding environment. Care should be taken when determining microplastics in species that feed via chemosensory sensilla as similar differences may be noted. A similar shortcoming in the use of freshwater macroinvertebrates as bioindicators has been noted previously as selective ingestion and egestion may bias the particle size distribution recovered, thereby not giving a true representation of microplastics in the environment (Ward *et al*. 2019). Additionally, as displayed by laboratory experiments (Mateos-Cárdenas *et al*. 2020) *G. duebeni*, can fragment microplastics during feeding thus changing the shapes and sizes of microplastics recovered which could result in differences in sizes and morphologies between those ingested and present in the surrounding environments. For example, the presence of films was noted in water samples but not in *G. duebeni* examined in this study.

The intertidal bivalve species *C. edule* displayed consistent microplastic concentrations (MP g^{-1}) throughout the study period and this stability between cold and warm seasons was likely due to the dynamics of the bay coupled with microplastic inputs from the freshwater environment. A wider range of polymers were identified in *C. edule* tissue than was observed in the sediment in chapter 4 and included the buoyant polymers PP and PE that were not identified in sediment. This species has potential as a bioindicator for microplastics given its ease of collection and wide geographical range. However, these results lead to the recommendation that it would be advisable to collect another species of bivalve that resides in the water column in addition to *C. edule*. As only a small proportion of microplastics identified in this species were polyethylene or polypropylene polymers despite their widespread use and production, it is likely that given its position in the marine environment *C. edule* will present denser microplastics residing in the bottom water compartment such as PA or polyesters more frequently than positively buoyant ones. As noted in chapter 5 of this thesis selecting more than one species when attempting to gauge microplastic pollution in the surrounding environment is likely a more appropriate method, however, the purpose of this study was to examine how these levels differed between seasons in this important species of bivalve. When attempting to understand microplastic presence in a deeper marine environment it is likely that viable results would be gathered by combining the sampling of a bottom dwelling bivalve in combination with one found in the water column as these would present more dense and buoyant polymers respectively.

The differences in microplastic concentrations (MPs g^{-1}) between the nine species examined when achieving research objective 3 of this thesis reaffirm the need to examine multiple species for microplastic pollution as one is not enough to gain a true perception of levels of contamination in marine ecosystems which has been noted previously (Valente *et al*. 2022; Pagter *et al*. 2020b). Further microplastic studies should therefore take a multi-species approach in assessing microplastic contamination.

In terms of future research goals for Dundalk Bay and its associated environs with regards to microplastic studies, several interesting routes exist. Sediment-burying bivalves have been documented displaying altered behaviours in sediment laden with microplastics in laboratory experiments (Urban-Malinga *et al*. 2021). An investigation to explore if similar behaviours occur in *C. edule* would be beneficial to understand the potential ecosystem-levels effects that the high levels of microplastics present in Dundalk Bay sediments may have on this environment, given the importance of this bivalve to the ecology and economy of this area. Further to this is the possibility of research into microplastics in the many species of seabird that reside in Dundalk Bay to assess the microplastic loadings likely occurring due to feeding on contaminated bivalves and other invertebrates as documented in this thesis. Laboratory experiments carried out on the *G. duebeni* recently have yielded important results displaying this species feeding on microplastic-contaminated food and fragmenting microplastics to smaller size (Mateos-Cárdenas *et al*. 2022, 2020). Further research could examine the propensity of this species to feed on a variety of coloured microplastics and chemical dyes that may trigger enhanced feeding rates and thus their potential strength as a biomonitor for microplastic in freshwater environments.

7.4 Policy recommendations moving forward

The presence of microplastics in Dundalk Bay and the freshwater environment associated with it add to the growing body of evidence that microplastic pollution is becoming pervasive in all environmental matrices. Worryingly microplastics have been documented in lungs, faeces, semen and placenta of humans (Zhao *et al*. 2023; Jenner *et al*. 2022; Yan *et al*. 2022; Ragusa *et al*. 2021). In spite of recent findings there is currently no regular environmental monitoring of this pollutant conducted in Ireland, with the closest to such action being a failed Bill from Green Party Senator Grace O'Sullivan in 2016 which looked to monitor all microplastics in Irish water systems and ban certain microbeads (Sargent, 2019). The subsequent Microbeads Prohibition Act 2019 that was introduced fails to include monitoring of environmental microplastics, thus limiting microplastics action on a legislative basis to primary microbeads, which account for only a very small proportion of overall environmental microplastics as highlighted by the findings from research objectives 1 – 3 of this thesis. The monitoring of microplastic pollution should be undertaken in the Irish environment as evidenced by the results of this thesis with microplastic concentrations in sediment of Dundalk Bay currently twice the estimated safe loading levels (Everaert *et al.* 2018) likely impacting on the functioning of this ecosystem.

While the EU Marine Strategy Framework Directive (2008/56/EC) addresses marine litter under descriptor 10 in its goal of achieving good environmental status (GES) it neglects the issue of microplastics. Additionally, there exists no current policies for the monitoring of plastic litter in freshwater environments despite the fact that as much as 80% of marine litter stems from the terrestrial environment (Mansfield *et al.* 2024; Hurley *et al.* 2020; da Costa *et al*. 2016; Jambeck *et al.* 2015). Furthermore, the third cycle of the River Basin Management Plans (RBMPs) is overdue for publication. The RBMPs set out goals for the protection of the aquatic environment and strategies for achieving them and is implemented in all EU countries under the EU Water Framework Directive. There also exists a gap between EU and national law on water quality and what is currently implemented at a ground level by local authorities. A recent EPA report detailed six local authorities failed to meet the required standard in each of the five water National Enforcement Priorities (NEPs) and there currently is no overarching local authority data gathering and reporting system for environmental enforcement in place (EPA, 2023). Due to these failings in current enforcement, it is unlikely that any specialised nationwide monitoring program for microplastics in either freshwater or marine environments is forthcoming in the near future. From the results presented in chapter 3 of this thesis and those examined through an extensive literature review, the freshwater environment is increasingly contaminanted with microplastic pollution with macroinvertebrates, the base of many food chains ingesting them in the natural environment with proven illicit effects documented in laboratory studies. Therefore, it is advisable that monitoring of microplastics occurs for water bodies concurrently with other monitoring parameters that are already in place to ascertain contamination levels nationwide.

Given the results presented in this thesis highlighting the ubiquitous presence of microplastics in the freshwater and marine environments and biota studied coupled with past studies conducted in Ireland (e.g., Murphy *et al*. 2022; Pagter *et al*. 2021; Mendes *et al*. 2021; O'Connor *et al*. 2020) it is likely that microplastics are pervasive in the majority if not all ecosystems nationwide. Due to their small size, it is not realistic to remove microplastics from the environment once they have been released especially as they can be broken down to form nanoplastics which have increased bioavailability thereafter (Monira *et al*. 2023; Zhao *et al*. 2023). Furthermore, the removal of macroplastics or microplastics from the environments fights the symptoms not the cause and fighting waste pollution should also fight waste production. In light of these factors and the lack of consistent monitoring for this pollutant, preventative measures should be encouraged to reduce their entry into the environment through a reduction in overall plastic use in general, however, plastic use is trending upwards in the EU currently. The findings from chapter 6 underline this need for preventative measures as the juxtaposition of the awareness and behaviours of stakeholders in Irish fishing was examined. Those surveyed generally viewed plastics as damaging to the environment, however, persisted with its use due to a lack of alternatives available while citing a lack of facilities and information on how to recycle obsolete equipment in a sustainable manner. Increased information to improve the circularity of Irish fishing should be promoted while research into alternative material should be incentivised which in turn would reduce plastic use and its entry into the environment and subsequent microplastic formation.

Between 2011 and 2021, the amount per capita of plastic packaging waste generated in the EU increased by 26.7%. While plastic production is expected to triple by 2060 the setting up of the High Ambition Coalition to End Plastic Pollution advocates for an ambitious and effective treaty that covers the entire plastics lifecycle to end plastic pollution by 2040 (European Commission, 2023) and is a positive step. At a domestic level there seems to exist a misalignment with stated environmental targets and laws and with actions that are taken by government. While bans on single-use plastics such as cotton-bud sticks, cutlery, polystyrene containers and straws came into effect from Directive (EU) 2019/904 in July 2021 (Single Use Plastics Directive) and the Microbeads (Prohibition) Act 2019 will reduce the amount of primary microplastics that enter the environment, no legislation has been adopted for the reduction in secondary microplastics, namely microfibres which are the most common found in the environment and stem from textiles (Herweyers *et al*. 2020). As was noted in the results of this thesis, stakeholders in Irish fishing were not aware of fibrous microplastics yet they consisted of the majority recovered from Dundalk Bay and its environs thus representing a knowledge gap possibly due to an underreporting of this type of microplastic in media in general. The fast fashion industry represents a source of synthetic microfibres to the environment with the International Union for Conservation of Nature (IUCN) calculating that 35% of microplastic pollution in the ocean stemming from the washing on synthetic textiles (Brodde 2017; Boucher and Friot, 2017). Further amplifying this problem is the aforementioned media focus which has been centred of microbeads giving less attention to microfibres which stem from these materials limiting the awareness of this issue in the general public which is needed to lead to a change in consumer behaviour. This is particularly important as the general public does not possess the ecotoxicological knowledge that scientists have. The findings from chapter 6 of this thesis highlighting that stakeholders in Irish fishing are unaware of fibrous microplastics was also recognised in a survey in consumer research conducted in the U.S.. The findings of that survey noted that adults who are familiar with microplastic pollution are most likely to identify plastic bags (76%) and microbeads (61%) from health and beauty products as contributors to microplastic pollution rather than microfibres stemming from clothing (Pope, 2023). The results of chapter 6 highlighting the lack of information stakeholders in Irish fishing had on microfibres, concurrently the most commonnly found in both the freshwater and marine environments examined in this thesis coupled with the vast majority of the literature detailing micofibres as the most prominent microplastic type in the environment underlines the knowledge gap that exists between scientific findings and the public. The dissemination of information pertaining to plastics and microplastics should be at the forefront of efforts to reduce plastic pollution levels as awareness is needed in order to lead to behaviourial shifts and greater efforts should be made by the scientific community and governments in sharing research outcomes on this topic.

Awareness and education on products that create microplastics should be at the forefront of reducing the quantities entering the natural environment. Unfortunately, Ireland is heading in the wrong direction with regards to tackling microfibre plastic pollution. The recent welcoming and official opening of ultrafast fashion brand SHEIN office in Dublin by the Minister for Enterprise, Trade and Employment Simon Coveney is a misstep in reaching our environmental goals and represents a direct contravention of Sustainable Development Goals (SDGs); 11 (sustainable cities and communities) and 12 (responsible consumption and production). It is also opposite to SDG 14 (life below water) and SDG 15 (life on land) while being indirectly misaligned for achieving SDG 10 (reduced inequalities) and SDG 13 (climate action) (UN, 2015). In order to reduce the volumes of microfibres entering the natural environment a reduction in the production of synthetic clothing is needed, however, this is unlikely to happen soon as unpopular policies are rarely adopted by Governments (i.e., removing cheaper clothing made from plastic polymers from shelves). The disproportionate focus and emphasis placed on microbeads and their removal was a relatively easy and welcome fix while targeting generally cheaper clothing to reduce microfibre release to the environment would for the most part not be supported by a generally uniformed public.

Unlike other challenges the impacts of microplastic pollution are not directly coupled to societal benefit and there exists only a finite amount of attention which can be diverted to other causes (Dr. Richard Thompson speaking in Paris at Limnoplast 2023). The scientific community, governments and media have a duty to better inform the public of the effects associated with microplastics and the combined problems associated with the overuse of plastics in general as current trends of plastic use increase, especially in Ireland. Currently microplastics emission to the environment is a secondary benefit of incentives to reduce waste creation. For example, the 2023 phasing out of single-use coffee cups in the town of Killarney, Ireland which generated 23,000 cups per week previously has reduced their waste output by 18.5 tonnes of waste annually (Carroll, 2023). On a larger scale the 2024 Olympic Games held in Paris will be the first major event without single-use plastic and visitors to the temporary competition sites in the French capital will only be admitted without plastic bottles (Reuters, 2023). As consumers, the scope for action is limited with personal and structural factors keeping consumers from reducing plastic pollution (Wiefek *et al*. 2021) as was noted in fishers surveyed as part of this thesis and the lack of alternative non-plastic gear. Further to this, the results of a representative survey which found that 87% of Europeans worry about the effects of plastic on the environment (European Commission, 2017). The onus therefore, should be on governments to pressure companies to reduce and take control of their plastic production and waste creation. An example of which is the recent lawsuit by the state of New York against PepsiCo over the plastic pollution in Buffalo River following a survey of riverine plastic litter found that 17.1% were produced by the company (Nerkar *et al*. 2023). It has also been suggested that warning signs on plastic products of the harm they can cause similar to warnings that appear on cigarettes should be implemented in order to curb the current unsustainable use of plastics that currently exists (Luo *et al*. 2022). A step in this direction has been adopted by the clothing company Levis with the sentence "*Releases plastic microfibres into the environment during washing*" included under the "Composition & Care" section of clothes made of synthetic polymers. Further measures could be the introduction of filters on clothing websites in order to shop by material in the same vein as filters such as length, size, colour etc. This could be a particularly powerful tool for use in Ireland given the huge per capita plastic waste that is generated, nearly double the EU average standing at 61kg (Murray, 2023). While just under 28 per cent of plastic packaging generated in Ireland was recycled in 2021 with the country set to miss mandatory EU recycling targets due to apply from 2025 for municipal waste, packaging waste and plastic packaging waste (EPA, 2023).

There exists a gap between intention and behaviour with regards to environmental action amongst the general public in Ireland. A recent Deloitte study of 1,000 Irish consumers found that while 49% stated they were willing to take action or change their behaviour with regards to climate action only 41% stated that they had purchased sustainable goods in the last 4 weeks namely due to the perceived high cost of such goods (Deloitte, 2023). This "say-do" gap was also evidenced in the results of this thesis wherein surveyed fishermen described the cost being a limiting factor for recycling used fishing plastics as well as a driving factor for plastic selection despite the fact that there was some acknowledgement that fishing was a source of plastic pollution to waterbodies. The recent introduction of the Return Scheme for recycling cans and plastic bottles at supermarkets and the return of a deposit included in the item cost originally will hopefully improve recycling rates for this sort of item throughout the country however it terms of microplastic pollution prevention it is quite literally a drop in the ocean. As evidenced by the results of chapters 3, 4 and 5 of this thesis fibres were the dominant microplastic type found likely stemming from the washing of textiles (polyamides / nylons and polyesters). While reducing the stem of secondary microplastics into the environment from the fragmentation of larger visible plastic items can be achieved via effective waste management systems, beach cleans and improved recycling facilities, these measures are largely ineffective for the trapping of microfibres. While microfiber emissions to the environment can be reduced on an individual level through the purchasing of eco-friendly, non-plastic clothing items it is unlikely to yield the type of reduction on the large scale necessary to protect the environment. I believe that appropriate legislative measures are the best method to prevent microfiber pollution. This could include a phasing-out timeline for clothing companies operating in Ireland to stop the use of plastic in their clothing ranges rather than some form of tax on these types of goods as again the cost would be likely shifted onto the consumer to cover. Improved media coverage on microplastic issues and on the overuse of plastics in general are also necessary as their consumption far outstrips measures to control their emission into the environment as well as warning symbols on these products as we know that plastics both increase greenhouse gas emissions and contribute to loss of biodiversity. As plastic production is expected to increase in the future urgent action is needed to reverse this trend and this should begin with greater information dissemination to the general public and to relevant government departments to "deplasticise" the world.

In the face of seemingly ubiquitous microplastic contamination of both biota and the environment the words of French philosopher Roland Barthes attending a plastics exhibition in the mid-1950s ring true today: "*The hierarchy of substances is abolished: a single one replaces them all*," concluding, "*the whole world can be plasticised*".

7.5 Take-home recommendations for future studies of this nature

- The standardisation of microplastic research is necessary to improve result comparability between studies, in terms of; controls, sampling, and units.
- Research should take a holistic multi-compartment approach rather than examining one environmental matrix in isolation as spontaneous contamination with microplastics does not occur.
- Microplastics research should be conducted in as an environmentally benign approach as possible especially for density seperations where less

environmentally harmful approachs are possible, in turn this will improve study comparability with citizen science projects.

- Greater effort is needed to improve consumer awareness of microplastics and the harm that overuse of plastics causes as an unsustainable resource.
- Engagements with stakeholders in fishing activities should be encouraged in order to look for solutions to this problem as this is likely the group that will see the greatest impact of microplastics.

Chapter 8: Appendices

Appendix A: Supplementary material for chapter 1

Recovery Rate Testing

Potassium carbonate recovery was tested using 6 different common polymers; R-PET, LDPE, HDPE, PS, PP and PVC. 10 microplastics from each polymer were added to samples of sediment and mixed in and extractions were carried out twice and this was repeated 3 times to generate mean recovery rates per extraction.

All microplastics were generated from "naturally" aged plastic items.

- A 5-year old credit card was cut up to create PVC fragments.
- HDPE fragments was created from a plastic petri dish
- LDPE film was cut from a plastic bag for plastic petri dishes.
- PS beads were taken from a bean bag and were placed in standing water and exposed to natural weather conditions of the study area for a period of 3 months.
- PP fragments were taken from an old plastic flower pot which had been stored in a greenhouse for a year previously.
- Recycled PET fragments were taken from packaging from supermarket-bought tomatoes.

Table 8-1: Recovery rates for various polymer types using potassium carbonate for density seperation.

Polymer Type	Extraction #1	Extraction #2
PET fragments	76.6%±5.7	93.3%±5.7
LDPE films	76.6%±5.7	86.6%±5.7
HDPE fragments	76.6%±5.7	93.3%±5.7
PS beads	80%±10	96.6%±5.7
PP fragments	80%±10	93.3%±5.7
PVC fragments	86.6%±15.3	100%±0

Resilience testing: No particle loss or deformation was noted following 30% H_2O_2 at 40°C following 24 hours of treatment and they were cleaned of attached debris, notably, the environmentally exposed PS beads which were fouled before the treatment.

Microplastic pieces of PET fragments, LDPE films, HDPE fragments, PS beads, PP fragments, PVC fragments and polyester fibres were added to 10 litres of Milli-

Q water with small sticks, leaves, silt and mud added to mimic the material collected in surface water. As mentioned by Walkingshaw *et al*. (2022) fibres with lengths greater than the mesh sizes were found on each sieve. For example, fibres with length >2000µm were found on the 50µm sieve having passed through the sieve stack before this level. This would indicate that fibres may potentially be lost due to sieving having passed through all sieves in a cascading sieve stack.

Raman analysis was conducted on microplastics recovered from blanks for all sections pertaining to microplastics work (chapters 3-5) and Polyethylene and cellulose were commonly noted amonst those identified, while cellulose may have stemmed from the 100% cotton labcoats it is unclear where the laboratory-introduced-polyethylene to controls originated.

Table 8-2: Contamination controls for sampling campaign 1.

Location	Hydrogen Peroxide Treatment and Centrifugation Contamination	Air Contamination	Total Laboratory Induced Contamination
Ramparts Headwater	2	0	2
Ramparts Outflow	0	0	0
Flurry Headwater	1	0	1
Flurry Outflow	3	0	3
Dee Headwater	2	2	4
Dee Outflow	2	1	3
Fane Headwater	0	0	0
Fane Outflow	0	1	1
Big Headwater	0	0	0
Big Outflow	0	0	0
Glyde Headwater	1	0	1
Glyde Outflow	1	1	2
Castletown Headwater	2	0	2
Castletown Outflow	2	0	2

Sampling Campaign 1: Contamination Levels for samples, Mean: 1.5 ± 1.29

Table 8-3: Contamination controls for sampling campaign 2.

Location	Hydrogen Peroxide Treatment and Centrifugation Contamination	Air Contamination	Total Laboratory Induced Contamination
Ramparts Headwater	0	0	0

Ramparts Outflow	2	0	2
Flurry Headwater	3	0	3
Flurry Outflow	2	1	3
Dee Headwater	0	0	0
Dee Outflow	1	0	1
Fane Headwater	1	0	1
Fane Outflow	0	0	0
Big Headwater	0	1	1
Big Outflow	0	1	1
Glyde Headwater	0	1	1
Glyde Outflow	0	3	3
Castletown Headwater	1	2	3
Castletown Outflow	0	1	1

Sampling Campaign 2: Contamination Levels for samples, Mean: 1.43 ± 1.16

Table 8-4: Labratory introduced contamination of *G. duebeni* samples.

Headwater samples	2 fibres
Outflow	1 fibre

G. duebeni Controls: Contamination for headwater samples: 0.3 Microplastics per composite sample

G. duebeni Controls: Contamination for outflow samples: 0.1 Microplastics per composite sample

Table 8-5: Labratory introduced contamination of sediment samples.

Headwater samples	2 fibres
Outflow samples	2 fibres

Sediment Controls: Contamination for outflow / headflow samples: 0.5 microplastics introduced per replicate.

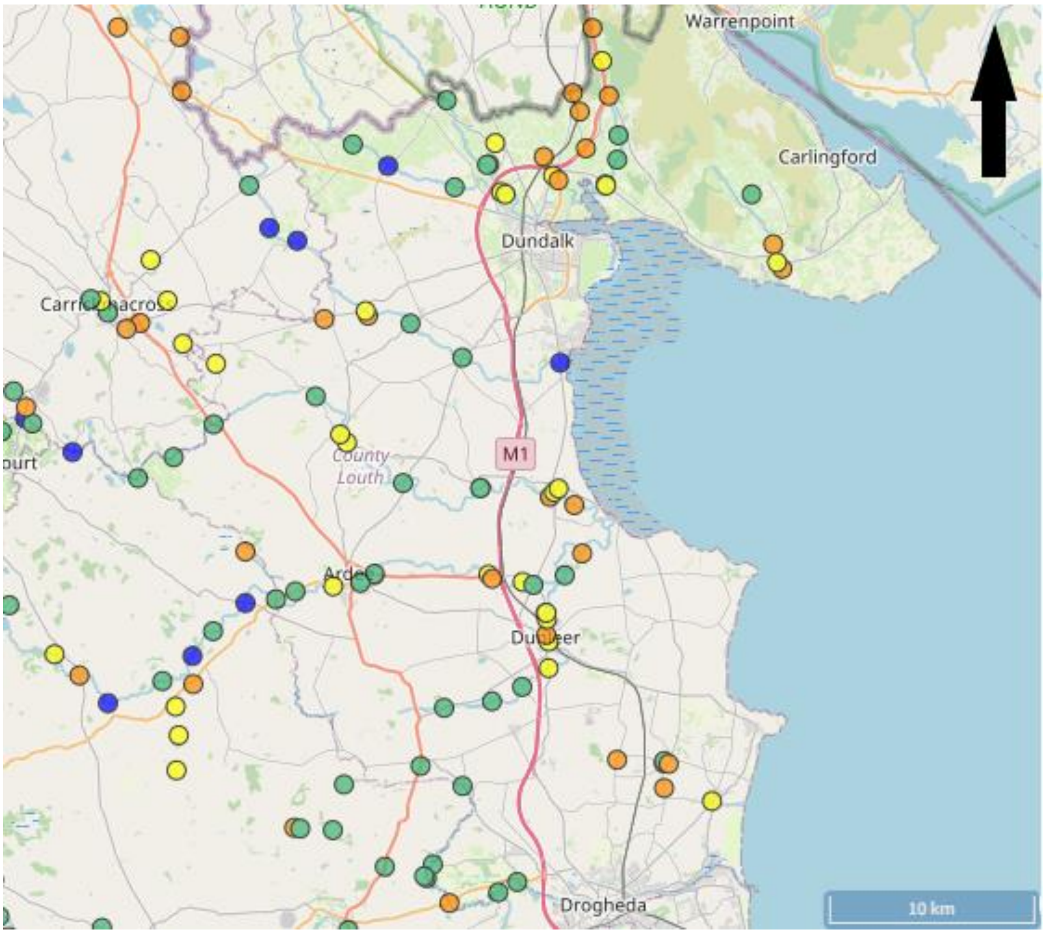

Figure 8-1: Map displaying Q-values of rivers entering Dundalk Bay. (EPA, https://gis.epa.ie/EPAMaps/).

Appendix B: Supplementary material for chapter 2

Sea-Fisheries Protection Authority authorisation for collecting cockles.

AN tÚDARÁS UM CHOSAINT IASCAIGH MHARA | SEA-FISHERIES PROTECTION AUTHORITY

Headquarters, Park Road, Clogheen, Clonakilty, Co. Cork, Ireland

T +353 (0) 23 8859309
F +353 (0) 23 8858796
W www.sfpa.ie

29th January 2020
DSR 02/2020

Dundalk Institute of Technology
Dundalk
Ireland

DEROGATION TO CONDUCT FISHING FOR SCIENTIFIC RESEARCH

Dear Dr Linnane

Please note that the Sea-Fisheries Protection Authority is pleased to agree to your request for a specific derogation to conduct fishing for scientific research subject to compliance with the terms outlined below;

Vessel Details: Name: **No vessel, collection by hand.**

Nature of trial: PhD scientific research under DkIT

Type of sampling: Sampling by hand.

Species being targeted: Cockles

Area coverage: Dundalk Bay

Period: January 2020-2022(dependent on weather/conditions/sampling success)

Scientific Staff: Stephen Kneel, Dr Caroline Gilleran Stephens, Dr Suzanne Linnane, Dr Alec Rolston.

Please be advised that a copy of this document should be retained while they are engaged in the scientific work.

Finally, I would like to wish you and your team every success with the project.

Christopher Nalty
Sea-Fisheries Operations Manager
cc: [Naval Service, SFPA-SMT, SFPA-Senior Port Officers]

1

Procedural blanks for marine beach sediment; Oil, ethanol and water.

Table 8-6: Contamination of procedural blanks for marine beach sediment: oil, ethanol and water.

Blank	Microplastics found
Rockmarshall Blank 1	1 fibre
Rockmarshall Blank 2	Clean
Rockmarshall Blank 3	2 fibres
Rockmarshall Blank 4	3 fibres
Rockmarshall Blank 5	1 fibre
Rockmarshall Blank 6	Clean
Rockmarshall*	1.16 ± 1.16
Blackrock Blank 1	Clean
Blackrock Blank 2	2 fibres
Blackrock Blank 3	3 fibres
Blackrock Blank 4	1 fragment
Blackrock Blank 5	2 fibre
Blackrock Blank 6	1 fibre
Blackrock*	1.5 ± 1.04

Table 8-7: Controls for cockle processing.

Season	a	b	c
Spring	1 fibre	1 fragment, 1 fibre	1.03
Summer	2 fibres	1 fibre, 1 fragment	2.07
Autumn	4 fibres, 1 fragment	1 fibre	1.11
Winter	1 fibre, 1 fragment	1 fibre, 1 fragment	2.05

Controls for cockle processing. a = air contamination, b = solution and c = potential contamination / cockle

Appendix C: Supplementary material for chapter 3

Table 8-8: Estimated processing-introduced contamination for each species.

Species	Air Control Contamination per individual	Solution control contamination per individual	Total Contamination per individual
E. siliqua	0.2	0	0.2
A. aculeata	0	1	1
E. cordatum	0.05	1	1.1
L. depurator	0.625	1	1.63
P. catenus	0.095	2	2.1
C. crangon	0	2	2
P. platessa	0.05	2	2.1
L. conchilega	0	1	1
Gobiiformes	0	2	2

Appendix D: Supplementary material for chapter 4

Ethics Approval for Survey issuance

DKIT
An Scoil Sláinte & Eolaíochta
School of Health and Science

2nd June 2020

Mr. Stephen Kneel,
Centre for Freshwater & Environmental Studies,
School of Health and Science,
Dundalk Institute of Technology,
Dundalk,
Co. Louth

Re: Microplastics in Dundalk Bay.

Dear Stephen,

The School Ethics Committee reviewed the ethics application for the above study at its meeting dated the 30th April 2020.. I acknowledge the amendments which you sent on the 29th May 2020. This application is now approved.

Wishing you the best of luck in your research.

Yours Sincerely,

Edel Healy

Dr.Edel Healy
Chair of School of Health & Science Ethics Committee

cc. Dr. Suzanne Linnane/ Dr. Caroline Gilleran Stephens/Dr. Alec Rolston

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Research article

Examining awareness, attitudes and behaviours of stakeholders in Irish Fishing towards plastic[☆]

Stephen Kneel[a,*], Caroline Gilleran Stephens[a], Alec Rolston[b], Suzanne Linnane[a]

[a] Centre for Freshwater and Environmental Studies, Dundalk Institute of Technology, Dundalk, Co. Louth, Ireland
[b] Goyder Institute for Water Research, Adelaide, South Australia, Australia

ARTICLE INFO

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Aquatic pollution
Plastic
Microplastic
Fishers' behaviour
Marine

ABSTRACT

This paper explores the awareness, knowledge, attitudes and behaviour of members of the Irish fishing community towards environmental topics such as; microplastics, plastic pollution and recycling. We conducted a mixed method survey consisting of 26 questions (2021) involving members of the Irish fishing community (fishers, aquaculturists etc.). Respondents were generally aware of microplastics and the threats they can pose to different environmental matrices. They noticed litter frequently when engaged in their fishing activities (0% never noticed litter) and in large quantities (35% of respondents noticed over 10+ items) but they were likely (likely 40% and highly likely 35%) to remove it from the environment. Durability was the main reason for the selection of most fishing plastics used by respondents (ranked first in 4 of 5 plastic items) while recyclability played a lesser role. Respondents also viewed plastics as cheap and convenient with these terms accounting for 48% of positive connotations related to the word 'plastic', however, in general associated plastic with negative phrases. Barriers to the recycling of used fishing plastics were most frequently identified as being due to a lack of knowledge on how to or a lack of facilities. This study provides novel insight into a previously unstudied cohort in Irish society towards plastics and recycling and can serve as guidance for further work on this group.

1. Introduction

Marine litter, especially in plastic form, is a pollutant of increasing environmental concern with around 8 to 10 million metric tonnes entering the ocean annually (Smith and Vignieri, 2021). Ghost-fishing from discarded or abandoned fishing gear (ghost gear) can be dangerous to marine life. For example, 'ghost' nets, can catch a large number of marine organisms attracting other creatures which in turn become entangled in a process known as cyclic catching (Havens et al., 2008; Link et al., 2019). Plastic's lightweight, durability and resilience to degradation creates ecological issues when released into aquatic environments namely marine wildlife can mistake plastic waste for prey with most then dying of starvation as their stomachs fill with plastic (IUCN, 2022). Macroplastics (>5 mm) (SAPEA, 2019) such as nets, bottles and other larger pieces of debris can interact and potentially harm marine life. Microplastics are particles of plastic which have an upper size limit of 5 mm (Auta et al., 2017; Anderson et al., 2016; Li et al., 2015). The number of marine organisms that interact with microplastics is likely many times higher than the approximately 700 species that are known to interact with larger marine debris (e.g through ingestion and entanglement) (Gall and Thompson, 2015; Gregory, 2009). Additionally, microplastics have been shown to be present in commercially important species consumed by humans worldwide such as oysters, mussels, herring, mackerel and tuna etc. (Van Cauwenberghe and Janssen, 2014; Rochman et al., 2015) and also in prawns found in Irish fishing grounds (Joyce et al., 2022).

The Republic of Ireland (ROI) has a poor record of plastic waste management, rated as the fifth worst country in the European Union (EU) at recycling plastic packaging recycling just 28% of it below the EU average of 38% (Eurostat, 2020). Additonally, almost two-and-a-half times more plastic packaging waste was sent for energy recovery in 2020 than was recycled (EPA, 2022). Plastic waste is also prevalent in the natural environment with, 91% of studied subtidal and intertidal Special Areas of Conservation (SACs) and Special Protected Areas (SPAs) in the ROI contaminated with microplastics (Mendes et al., 2021).

Plastic waste in the ocean is a major environmental threat and accounts for 85% of marine litter (UN Environment Programme, 2021). By the year 2050, it is estimated that the mass of plastic debris in the oceans will surpass that of all fish species combined (Jambeck et al., 2015). This projection may become reality sooner due to the

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* Corresponding author.
E-mail address: stephen.kneel@dkit.ie (S. Kneel).

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increased plastic material in circulation as a result of the disposable face masks, components of antigen tests and also increased use of PPE in hospitals from the COVID-19 pandemic (Peng et al., 2021). Irish coastal locations that are polluted with more than one disposable face mask has increased from 18% of 710 surveyed locations in 2020 to 21% of the same locations in 2021 (Coastwatch Europe, 2021). Simulations from 24 sampling expedition's estimate that around 268,940 tonnes of floating marine litter are in the ocean ranging from microplastics (<4.75 mm) to macroplastics (>200 mm) equating to the equivalent of 5.25 trillion pieces of plastic (Eriksen et al., 2014). There is a discrepancy between the marine biomes surveyed for marine litter. Most global studies have typically focused on floating debris on the surface layer of the ocean and stranded litter on coastal beach locations (Madricardo et al., 2020), while the seabed has been comparatively understudied (Debrot et al., 2013; Rizzo et al., 2022) in part due to the difficulty in accessing this environment. Some areas of seafloor around Europe's coasts can have up to 10,000 plastic items per hectare (Thompson et al., 2009), suggesting a less tangible but still very real threat to fisheries from plastic litter. This is particularly problematic given that microplastics have been documented in shellfishes sold for human consumption (Daniel et al., 2021).

The economic cost of marine plastic litter to nearly all marine ecosystem services should not be underestimated. Using 2007 US$ values Beaumont et al. (2019) estimated economic costs per tonne of plastic litter in the Ocean to services such as fisheries, recreation and heritage were estimated as $3300-$33,000. Furthermore, early studies of commercial and subsistence fishers found a largely negative view of marine litter which they associated with; fouling, damage to gear and propeller entanglement which affected their catch as well as posed a safety hazard (Nash, 1992; Wallace, 1989). Fishers may also be impacted economically through microplastic impacts on fish species. Intestinal blockage, physical damage, histopathological alterations in the intestines, change in behaviour, change in lipid metabolism and transfer to the liver are some observed effects of microplastics ingestion (Jovanović, 2017). The Irish Seafood industry employs nearly 16,000 people either directly or indirectly, has 1993 registered vessels, 296 registered aquaculture sites and is worth approximately 1.3 billion euro (BIM, 2022). Furthermore, there exists dozens of angling societies and clubs across the country enabling people to take part in a leisurely outdoor activity which rely on stable fish populations (Links to Irish Angling Clubs and Associations, 2022).

The ROI has taken steps to limit the introduction of plastic waste to the environment and also to encourage its removal. These actions include; the introduction of legislation relating to banning microbeads, introducing levies on plastic bags in shops and the establishment of Bord Iascaigh Mhara's Fishing for Litter scheme in 2015 which is part of a European wide initiative. The ban on the sale of certain single use products (e.g. straws, stirrers, cutlery) came into force into July 2021 and soft plastics have been reintroduced to the ROI's recycling list since September 2021. Over the last few year's legislation regarding proper plastic waste management have been implemented e.g. Delegated Regulation (EU) 2020/2174. At national level, the waste action plan of the ROI (2020–2025) intends for packaging on the Irish market to be reusable or recyclable by 2030 and introduce a deposit and return scheme. As the first country in the EU (following the United Kingdoms departure) to ban microbeads under the *Microbeads (Prohibition) Act 2019 (S.I. No. 36 of 2020), art. 2* which came into effect on the 20th of February 2020, the ROI was at the forefront of action against microplastics. However, this ban does not include products that protect from UV light such as sunscreen which are still permitted to contain microbeads. Microbead-containing sunblocks may be washed from people swimming in the marine environment, directly entering coastal ecosystems. Furthermore, no such action has been taken against products which can shed microplastic fibres such as polyester clothing which are regarded as one of the main microplastic pollutants in the environment (Herzke et al., 2021; Acharya et al., 2021; Cole, 2016; Gago et al., 2018).

One success story is the aforementioned Fishing for Litter initiative in ROI (BIM, 2023) which was established in 2015 with the support of the European Maritime and Fisheries Fund. The scheme encourages commercial fishing ships to return any waste material to shore that they might pull in during their fishing activities rather than discarding back overboard as might have been the case in the past. By the end of 2019, 95% of trawlers (244 boats/vessels) operating from the ROI's 12 main fishing ports had joined the programme. Large hardwearing bags are given to trawlers for the collection of waste they collect from fishing and when full are moved into a designated skip by harbour staff. To date 409 tonnes of marine litter have been retrieved via this initiative (BIM, 2023).

In order to understand the pathways of plastic waste and microplastics as breakdown products entering the environment and reduce those quantities it is crucial to understand the role that humans play in the process. The human dimension in plastic pollution and microplastics is threefold. Namely; plastics are entirely anthropogenic in nature, microplastics can have a negative impact on humans (ingestion of microplastics in diet, debris littering areas of natural beauty etc.) and humans can help to address the problem (Pahl and Wyles, 2017).

While studies have been carried out in the past on the knowledge levels, attitudes and awareness of the public towards topics such as marine litter, marine threats, plastic pollution and microplastics research gaps still exist. Very little work has been carried out on understanding these aspects in a group that may be directly exposed to issues around marine litter and in particular microplastic pollution, i.e. fishers. For example; stakeholder perceptions of marine plastic waste management in the United Kingdom was examined across 22 different groups including; marine advisors in government agencies, marine biologists, maritime researchers and enquiries officer of a marine NGO (McNicholas and Cotton, 2019), however, fishers were omitted from this study. Limited data is available on fishers and their perceptions with regard to topics surrounding plastics with one study examining fishmongers, commercial fishers and recreational fishers in a fishery in South Australia showing that all three groups misperceived plastic pollution as less of an issue locally than internationally (Wootton et al., 2022).

This knowledge gap needs to be addressed as the fishing industry is both a starting point of plastic entering the marine environment (lost fishing gear) and likely an industry that may be affected by microplastic pollution in the future as microplastics have been found in fishmeal for aquaculture and commercial fish species already (Thiele et al., 2021; Di Giacinto et al., 2023). Abandoned, lost, or otherwise discarded fishing gear (ALDFG) containing plastics accounts for another 27% of marine litter items found on European beaches (European Commission, 2018). It is unclear how much ALDFG enters the marine environment every year. The figure of 640,000 tonnes listed by Food and Agriculture Organization of the United Nations is based on a 1975 estimate and fails to account for the rapid expansion and modernisation of many fishing fleets across the world (Richardson et al., 2021). A median of 48.4 kt of fishing gear was lost during fishing that amounted to 74% of marine capture globally in 2018 (Kuczenski et al., 2022). The model did not include fishing gear that is abandoned or discarded intentionally. Approximately 18% of marine plastic debris in the ocean is attributed to the fishing industry (Andrady, 2011). Ghost gear is estimated to account for approximately 10% of plastic litter in oceans however they form the majority of macroplastics (>20 cm) by weight found floating at the surface (>70% by weight) (Eriksen et al., 2014). This larger fishing gear can break down to form thousands if not millions of microplastics both on shorelines and to lesser extent at the surface of the sea. While as much as 80% of plastic that ends up in the marine environment stems from terrestrial sources it is fishers and marine-based groups that must deal with the consequences of this pollution (Andrady, 2011). Post-mortems conducted on dead dolphins found washed ashore on the West Coast of the ROI attributed their deaths to entanglement with fishing gear (Healy, 2013). Given the dangers posed by ALDFG it is important therefore to understand the perceptions and attitudes that fishers have towards topics such as recycling, marine litter, microplastics and plastic. This in turn can lead to widely acceptable solutions to help mitigate litter inputs from fishing activities into the both freshwater and marine environments.

Fishers currently operate in a world with multiple stakeholders and the way society perceives environmental consequences of fishing is changing (OSPAR, 2018). Given the fact that fishing gear is an important source of litter entering aquatic environments and therefore microplastics coupled with microplastics presence being noted in many species of fish indicate that fishers are an important group to assess with regards to their attitudes, awareness and behaviour towards topics such as plastics, recycling and microplastics.

Understanding the views of Irish fishers on plastic pollution mitigation methods i.e. recycling as well as their behaviour towards litter they encounter during their fishing activities will help to develop effective strategies to hopefully reduce the leakage of fishing plastics into the aquatic environment. As noted by Wootton et al. (2022) understanding the perceptions of this group of important stakeholders have on plastic pollution will hopefully generate solutions that can effectively address this environmental issue that are more easily accepted by multiple stakeholders. The importance of studying the perceptions and attitudes to plastic and microplastic pollution cannot be understated as they are largely unexplored and deserving of more attention as it is human behaviour that leads to its occurrence in natural environments (Deng et al., 2020). With this in mind, the views of members of the Irish fishing community were examined in order to understand their perceptions, awareness and behaviour around plastics and associated topics. It is hoped that the findings of this work will aid in effective policy development and help to mitigate plastic pollution entering the aquatic environment of Ireland.

2. Research aims

As far as the authors are aware this is the first study of its type in the ROI which examines the awareness and knowledge levels, attitudes and behaviours of stakeholders involved in the Irish fishing community addressing the following research questions:

1. How knowledgeable and aware are Irish fishers about plastic and the impact of plastic waste on the environment?
2. What are the attitudes of fishers toward plastic use in fishing and recycling?
3. What behaviours do Irish fishers exhibit with regard to plastic waste?

3. Methodology

A mixed method survey was designed in order to answer the overlying research questions. Given that a deep understanding of fishers' attitudes and concerns was desired, the mixed method approach of a combination of not only qualitative but also quantitative data was selected as the best way to achieve this. Mixed method surveying allows the combining of the two aforementioned types of data which can compliment each other and enables real experiences in qualitative responses to be matched with quantitative data which helps to validate the results of the survey (Cresswell and Cresswell, 2018). The coupling of two different data gathering methods in this survey enable the real-world observations of fishers from the aquatic environment to be quantified and their interpretivism around subjective experiences and their own understanding of environmental topics such as recycling and microplastics to be expanded on (Wasti et al., 2022).

4. Survey development and participants

The survey was initially developed and refined by consultation within the authors' research group. The survey was then formally piloted with a random sample of fishers in September of 2020 trialling physical copies of the survey and minor modifications were made following this. Each respondent was asked 26 questions which included demographic information followed by questions addressing the awareness, knowledge, attitudes and behaviour of the respondents in relation to plastic pollution, plastic use in fishing and management of plastic waste. This study was conducted among individuals involved with fishing (both commercially and recreationally), angling, fish processing and aquaculture in the Republic of Ireland, during the period from May to November 2021. Those involved in scientific research relating to fisheries were also eligible to participate. Participants that did not match these criteria were excluded.

5. Sample size

The revised survey was administered online via google forms from May 2021 to November 2021 and distributed by email and social media platforms. To specifically target stakeholders involved in the Irish Fishing community the survey was published on the Inland Fisheries Ireland angling website (www://fishinginireland.info/2021/fishing-updates/contribute-to-research-into-plastic-pollution-in-our-waterways/) and emailed to subscribers of Irish Angling Update.

6. Ethics and consent

The research underwent a full, thorough, internal, institutional ethical review in Dundalk Institute of Technology and was conducted in accordance with Standard Operating Procedures (SOPs), adapted from the model SOPs developed by the Association of Research Ethics Committees (Association of Research Ethics Committees (AREC) 2013). No information was withheld and the minimal risks were clearly explained through informed consent. To ensure confidentiality, anonymity was assured at all times. All data were stored safely and securely in accordance with institutional policies. The ethics committee at Dundalk Institute of Technology approved the survey.

7. Data analysis

Data were cleaned and analysed statistically using the Minitab statistical software package (version Minitab® 21.1.1 (64-bit)). Descriptive analysis was conducted, and data were reported as percentage and frequency. Answers to the open ended; 'Please give your understanding of the term microplastic?' were graded and given a score out of six. Scores of '0-2' indicated no or little knowledge of the topic, 3–4 indicated an adequate understanding of the topic and scores 5–6 were given to answers which displayed a high level of understanding of the topic. The knowledge scores data were tested for normality using the Anderson–Darling test, which revealed it was non-parametric. Following this median knowledge score of microplastics vs the age of participants was examined for significant differences using Kruskal–Wallis test (significant if p value less than 0.05) using Minitab statistical analysis. Content analysis was carried out on open-ended responses and these were analysed in order to identify trends and themes and interpret the data effectively. Graphs were constructed using Microsoft Excel 2016.

Data regarding participant attitudes towards plastics and recycling was also collected. The terms were analysed through inductive content analysis (Vaismoradi et al., 2013) (Dilkes-Hoffman et al., 2019; King et al., 2023). Word themes/stems or synonyms were identified and then word frequency was determined for each identified word/phrase. A word cloud was constructed from all words reported by two or more participants using the freely available online program 'wordart' (http:

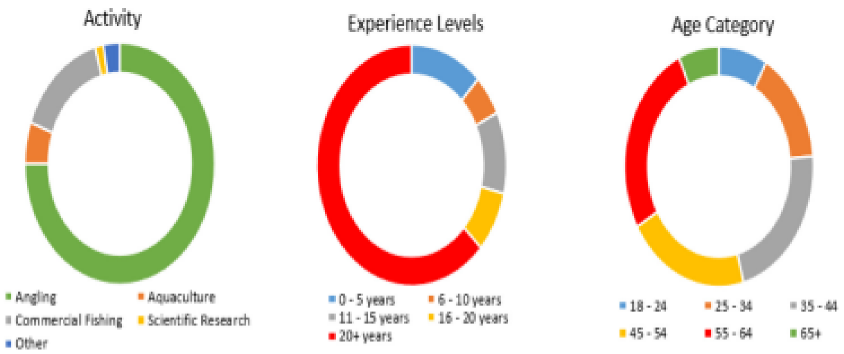

Fig. 1. Chosen activities, experience levels, and age categories of respondents. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

//www.wordart.com/). Font size was used to depict word frequency and font colour to represent negative, positive and neutral connotations. Red was selected to portray negative words/phrases, green for positive words/phrases and blue for words/phrases deemed to be neutral/ambiguous (Vrain and Lovett, 2020). Words were classed as negative, positive or neutral/ambiguous through individual assessment and post-assessment comparison between the authors (Dilkes-Hoffman et al., 2019).

8. Results & discussion

8.1. Demographic information

In total 73 responses were received to the survey (70 males and 3 females), after data cleaning, 72 responses remained for final analysis (70 males and 2 females). The demographic and activity breakdown of respondents are presented in Fig. 1 below. Although there was a lower amount of respondents than hoped for, the number of responses received is not dissimilar to that obtained when Wyles et al. (2019) surveyed the fishing community in the UK (n = 97) (all male). This small number of respondents can be due to the difficulty of reaching this specialised community (e.g. fishers being at sea sometimes 6–10 days at a time) (Wyles et al., 2019). From the authors' own experience communicating with this particular cohort can be difficult. Many fishers, especially older fishers have very little or no online presence (lacking social media or email addresses), however, an online only version was used following the pilot study results as when completing physical copies of the survey respondents were able to skip questions which led to patchy data collection with incomplete surveys being returned. The online survey required every question to be completed and so they had a 100% completion rate. The age profile of respondents skewed towards the older age categories (35–44≤), this is in contrast to the results of a meta-analysis of surveys which found that generally age has a negative relationship with response rates (Wu et al., 2022). Amongst respondents, 63% had been involved in fishing activities for more than 20 years which may bias data as not being representative of the Irish fishing industry as a whole (i.e. more traditional views and methods to handling litter may prevail). Considering that in 2017 women made up just 7% of those employed in the Irish seafood industry it was not surprising that 97% of respondents were male (UN Food and Argicultural Organisation, 2019). The majority of respondents (75%) selected angling as their primary fishing activity and 15% selected commercial fishing (Fig. 1).

Just over half of respondents selected their location as counties with coastline (53%) and with 66% reporting that they spent a considerable amount of time in the natural environment when partaking in their selected activity (once-a-week or more frequently). Additionally, 14% of responses were categorised as a non-specific Irish location and included terms such as 'Eire', 'Southern Ireland', 'South west', or 'all over the country' for example. These non-specific Irish locations likely refer to vessels that leave Irish coasts but travel far out to sea for fishing purposes.

8.2.1. Irish fishers have a high level of awareness on plastic pollution

A majority of those surveyed (68%) stated that they had noticed an increase in the use of plastics in their selected activity, 25% stated they had not while 7% said that they did not know. This may be due to the fact that over the past few decades there has been an increased reliance on plastic in fishing activities in the form of nets, lines, ropes etc., with the lightness, durability, buoyancy and low cost making it ideal (Watson et al., 2006; Andrady, 2011; Wootton et al., 2022). While overall there was a noted increase in the use of plastics in fishing activities amongst survey respondents this was not always the case when examined in relation to the experience level of respondents. The majority of those involved in their fishing activity for between 11–15 years stated they had not noticed an increase in plastic use in their activity, while half in the experience category of 6–10 years said they also had not. Conversely 75% of those in the '20+ years' experience category stated that they had noticed an increase while nearly 80% in the least experienced category of '0–5 years' also noticed an increase despite their relatively short time spent in their selected activity, this was however a smaller group than the aforementioned one (Supplementary material Appendix 1, Fig. A1).

Waste material seems to be ubiquitous in environments where fishing occurs in ROI. When fishers were asked 'when partaking in your selected fishing activity how often would you notice the presence of items that may be classed as waste material (e.g. plastic bags, water bottles, discarded fishing lines, etc.)?' 43% and 31% of respondents respectively stated 'Every time' or 'often'. A smaller portion consisting of 22% of those surveyed said they noticed waste material only 'occasionally' while the final 4% stated they 'rarely' did. Given than no respondents stated they never saw litter this would mean that 100% of those surveyed noticed litter when taking part in their fishing activity highlighting the ubiquity of litter in aquatic environments (Fig. 2). This is in line with other results that have examined similar topics. For example, the MARLISCO survey (3748 respondents from 16 European countries) found that most people reported that they noticed marine litter on most or every visit to the coast and that they said this situation was deteriorating (MARLISCO, 2015). While 81% of members of the public declared they witnessed plastic pollution on a 'daily' or 'weekly' basis in UK-based focus groups (Henderson and Green, 2020). In the current study, participants were also asked to numerate the amount of waste they normally notice when conducting their normal fishing related activity; 'when partaking in your selected fishing activity how

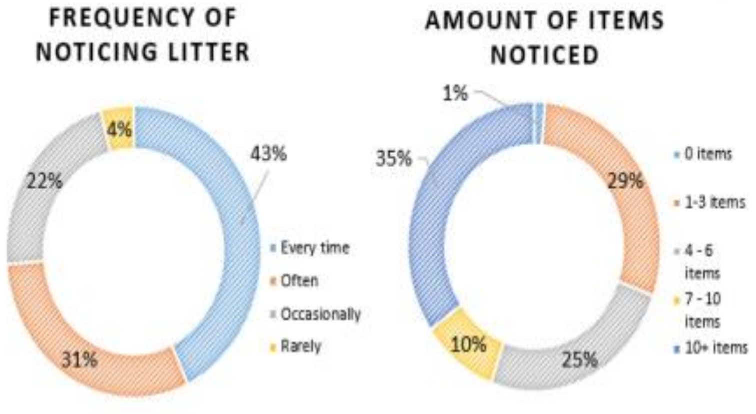

Fig. 2. Fishers interactions with litter in aquatic environments.

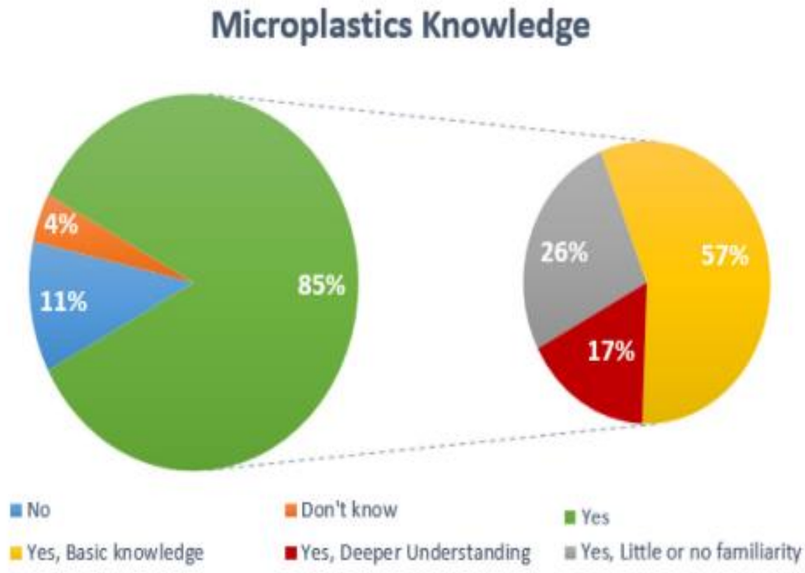

Fig. 3. The breakdown of respondents that had heard of the term microplastics before and their subsequent knowledge levels.

many items would you normally notice that may be classed as waste material (e.g. plastic bags, water bottles, discarded fishing lines, etc.)?'. The largest amount of responses fell into the '10+ items' at 35%. This was followed by; '1–3 items' at 29%, '4–6 items' at 25% and '7–10 items' at 10% of total responses. The category with the lowest amount of responses was '0 items' which had just 1 response (Fig. 2).

Fishers were found to have a high level of awareness on the topic of microplastics. Eighty five percent (85%) of respondents answered they had heard of the term microplastics previously with 11% stating they had not and 4% stated they 'don't know' with a further breakdown shown in Fig. 3. This is much higher than reported by focus groups on the general public in the UK where very few of the participants had heard of the term (Henderson and Green, 2020) and from UK members of the public where 68% of survey respondents did not know what microbeads were (Greenpeace, 2016). This may underline the fact fishers have a vested interest in this environmental pollutant and are therefore more aware.

Some examples of highest-scoring responses included: *'Break-down of plastic particles suspended in waters at a microscopic level'* or *'plastic which has been broken up into small pieces so it is no longer visible and very difficult to take out of the water'*. Examples of lowest scoring answers besides the null responses which earned 0 as a score include: *'microplastics are used in products such as face washes'* and *'in shampoo, etc.'*. Of survey respondents that gave a description to what they thought microplastics were; 33% mentioned a source (breakdown/designed) and included phrases such as; *'by-product from manufacturing'*, *'large plastic breaking down'* and *'found in shampoo'*. Additionally, 35% of responses included a 'location/area of effect' of microplastics and mentioned terms such as; *'small tiny plastic fragments, that fish usually eat'*, *'small plastics that plankton and aquatic life will feed on'* and *'microplastics are broken down particles of larger plastic pieces which can enter the food chain by being eaten by organisms or absorbed by organisms'*. Interestingly one respondent stated *'Microscopic plastics in water which impact fish and aquatic life. Most I've seen has been related to the ocean, not freshwater'*, indicating that they were more knowledgeable on the topic with regards to the marine environment than freshwater. This knowledge was noted in those surveyed as a whole and was further evident in their

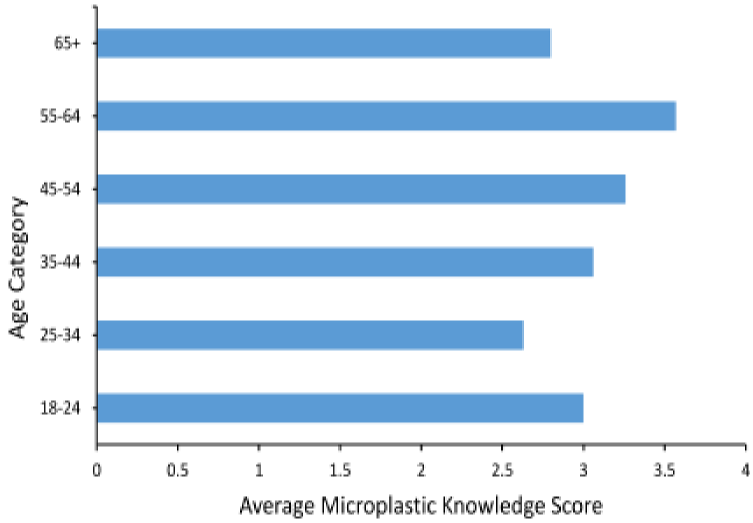

Fig. 4. Mean microplastic knowledge score for age categories.

responses to awareness of plastic pollution and microplastics in various environments (Supplementary material Appendix 1, Fig. A2).

Following this, the total scores of microplastic understanding and the mean score per respondent were tallied for each age group (Fig. 4). The age category group; '55–64' had the highest mean score for microplastic understanding amongst those surveyed at 3.57. The lowest mean score was seen in the oldest age category surveyed; the age category '65+' which had a mean score of just 2.8. The remaining 4 age categories (18–24, 25–34, 35–44, 45–54) all returned mean scores of microplastic knowledge of; 3, 2.63, 3.06 and 3.26 respectively. As data was deemed to be not normally distributed via the Anderson–Darling test for normality Kruskal–Wallis analysis was carried out. This analysis found that there was no statistical difference between the median knowledge values of each category ($P = 0.927$). The mean score for all age categories was 3.15 ± 1.66 which falls into the bracket of having basic knowledge of microplastics while the median score was 4 which falls into the same score bracket.

The understanding and awareness of the impact of plastic pollution and microplastics on various environments and aquatic life was measured using a Likert scale. Overall levels of awareness by fishers on all topics was generally quite high (Supplementary material Appendix 1, Fig. A2). Percentage responses in 'aware' and 'very aware' categories ranged from 57% for the topics; 'Microplastics on land' and 'microplastics in freshwater environments' to 89% for the topic: 'Plastic in the ocean'. This high level of awareness may be due to the media coverage of topics such as the great pacific garbage patch and footage of cetaceans interacting with plastic items in recent years. When assessing the same environment (oceans) on the topic of microplastic pollution awareness was still quite high with 75% of participants 'aware' or 'very aware'. This difference in awareness levels may be due to the fact that macroplastic or plastic pollution is a more tangible thing that can be witnessed with the naked eye in natural environments while in general microplastics will not be visible to participants in fishing based activities without searching for them or using specialised lab equipment. Despite these differences microplastic awareness was still quite high especially for the topic; 'Microplastics in oceans'. Recent emphasis in the media on research on these topics and television shows (e.g. Food Unwrapped: the plastic in our food, 2017, the Irish state broadcaster, RTÉ sharing news items on microplastics e.g. '*How wet wipes and sanitary products are causing marine pollution*' (Morrison, 2020)) discussing these topics may be creating this, perhaps unexpected, awareness amongst the cohort surveyed. This indicates an awareness among respondents about the negative effects of plastic on the environment, which is aligned with other studies (Filho et al., 2021a; Kershaw et al., 2011; Otsyina et al., 2018). This is reflected in the wordcloud (Fig. 6) generated where the majority (53%) of phrases associated with plastics were deemed negative. The potential impact of media, although unquantified in this study, cannot be understated as media help to simplify complex scientific issues and topics and present them in a 'storyline' format in which audiences can engage with moral responsibility and interpretation (Entman, 1993; Gamson and Modigliani, 1989). Furthermore, media outlets tend to sensationalise scientific findings in order to make a more exciting or attractive story and for example; 'microplastics in the human body' makes an attention-grabbing, scary or 'clickbait' headline (Dempster et al., 2022; Chakraborty et al., 2017; Völker et al., 2020). Additionally, the nature of global news and social media enables the blame and guilt associated with plastic use to be shifted from individuals to other groups (Wootton et al., 2022).

Overall, microplastics on land followed by microplastics in freshwater environments were the topics with the highest selection of 'not aware' at 25% and 19% respectively. The selection of not aware on the topic of microplastics on land was twice as high when compared to the selection of not aware on the topics of microplastics in oceans and the topic of microplastic pollution interacting with marine or riverine life (fish, insects, birds etc.) and links in with the understanding of the term microplastics of several respondents. While at first glance it may appear that fishers seem to be less aware of impacts or topics that they view as unrelated to the area they carry out their primary fishing activity in (i.e. terrestrial environment and microplastic impacts), the same was seen in Irish farmers who felt they knew more about microplastics and plastic pollution in aquatic environments than on land (King et al., 2023).

Respondents were asked to rank several different topics on how serious they view the threat of plastic pollution towards them (Supplementary material Appendix 1, Fig. A3). Overall, the perceived threat of plastic pollution was high; this ranged from 68% (serious or very serious) for the threat to 'humans' or 'land' to a high of 86% for oceans. This ranking of plastic pollution threat to the oceans as the topic with the highest amount of responses of 'serious' or 'very serious' is in keeping with the findings of Dilkes-Hoffman et al. (2019) that 69% of members of the public rated plastic in the ocean as very

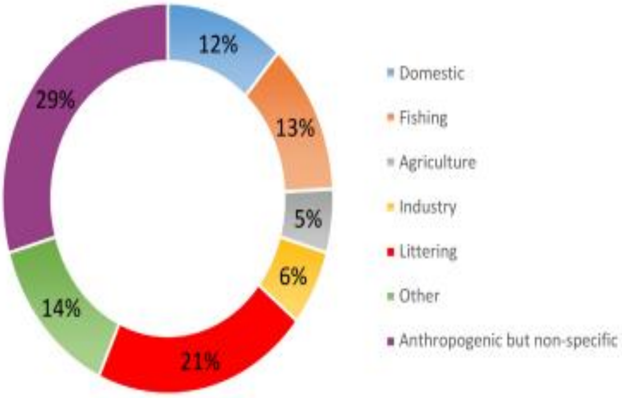

Fig. 5. Perceived plastic pollution sources of aquatic environments.

Fig. 6. Wordcloud displaying phrases fishers used to describe plastics.

serious. The topic with the highest percentage (57%) of responses in the 'very serious' category was 'freshwater and marine wildlife' and obtained 81% of responses in the combined 'very serious' and 'serious' categories. This likely reflects the fact that Irish fishers' livelihoods and recreational activities depend on the wellbeing of fish stocks now and in the future. The responses of this cohort of Irish fishers is in keeping with the views of the Irish public in general, as 85% were 'extremely concerned' or 'very concerned' about the impacts of plastic on ocean health and marine life (EPA — Plastics report, 2022). The threat of plastic pollution to humans while still perceived as high albeit reduced compared to the aforementioned topics. Receiving 68% of responses in this study in the 'very serious' and 'serious' categories was also similar to the responses of the EPA study of the general public on the 'potential human health impacts' which received 74% of responses in the 'extremely concerned' or 'very concerned' categories.

8.2.2. Irish fishers view plastic as convenient and cheap but damaging to the environment

The next part of the survey sought to understand fishers' attitudes towards plastic pollution and their general views of plastics. Responses to the following question; 'where do you think plastic pollution in rivers/the ocean is coming from?' were categorised into the following; domestic, fishing, agricultural, littering, other and anthropogenic but non-specific (Fig. 5) and sought to understand who Irish fishers' see as being responsible for plastic pollution of aquatic environments. Anthropogenic but non-specific was the most commonly seen response to sources of plastic pollution in rivers/oceans with 29% of responses falling in this category. Littering was the next most common response (21%) followed by; other (14%), fishing (13%), domestic (12%), and industry (6%). The category with the lowest percentage of responses was agriculture (5%). 'People' was the most common answer, mentioned 11 times in responses while 'humans' were mentioned 5 times. Anthropogenic sources i.e. 'humans' or 'people' were the most common answers. This would indicate that the majority of those surveyed understand that plastic pollution is a human problem they seem to place the blame at the foot of individuals i.e. 'careless leisure seekers' or 'lazy people' instead of larger sectors such as industry and agriculture which were the two least commonly seen responses. This would indicate that in the eyes of this cohort from the Irish fishing community a disconnect exists between the polluting of waterbodies and larger scale activities. Although blame for pollution of rivers/oceans was placed at the foot of individuals no respondents said that they themselves caused littering and instead described those that did cause littering as 'lazy' or those lacking in education; 'lack of education to bin properly'. This is in line with previous studies (e.g. Campbell et al., 2014; Santos et al., 2005; Slavin et al., 2012) that found despite generally claiming not to be responsible for littering themselves members of the public identified beach users as the main source of marine litter. This blame allocation for littering may stem from traditional schemes in the ROI where the emphasis for litter management and recycling has been placed at the foot of the public rather than at that of corporations. An example of which was the introduction of the 15 cent levy on plastic bags at the point of sale for consumers in the ROI (MARLISCO, 2002).

Given that members of the Irish fishing community surveyed recognised that fishing activities create plastic pollution in aquatic environments they may be open to novel methods to reduce this occurrence. An example on how to reduce the amount of ALDFG generated is via radio frequency identification (RFID) tags which can be installed on fishing gears such as buoys or highflyers of gillnets and longlines as gear marks. The only RFID technology test for fishing gear position marking was a pilot study by Irish Fisheries Board (BIM, 2007) and found that off-the-shelf commercial equipment had a range of approximately 240 m and may be useful for the recovery of lost gear where the general location is known (He and Suuronen, 2018). Furthermore, significant interest has been generated in ropeless trap/pot fishing due to an increase in humpback whale entanglements and the 2017 North Atlantic right whale unusual mortality event (Myers et al., 2019). A switch to more high-tech fishing gear and tags may lead to a reduction in ALDFG, which could be driven by the introduction of subsidies by government to fund this change as evidenced by the 'Ghost Gear Program' in (Fisheries and Oceans (DFO) Canada, 2021). The Ghost Gear Program funded by the Canadian government has supported 49 projects under four program pillars: (1) ghost gear retrieval; (2) responsible disposal; (3) uptake and piloting of technology to prevent gear loss; and, (4) international leadership (Fisheries and Oceans (DFO) Canada, 2021).

Another open ended question put to respondents; 'please list below two words/phrases you associate with plastics (either positive or negative)'. For ease of interpretation the results are presented as a word cloud (Fig. 6). All of the responses were categorised as either positive, negative or neutral. Terms with positive connotations made up 37% of the total comments with cost 'cheap' (n = 10) and convenience (n = 10) being the most common responses. The only other terms that were mentioned more than once were; 'recycle', 'durable' and 'essential' with the latter two terms relating to the functionality of plastics and durability a recurring theme from fishers selection of using specific fishing plastics. The majority of comments, however, were deemed to be negative (53%). General environmental concern (n = 39) ('damaging to the environment', 'environment killer'), association with litter and pollution (n = 7) ('unsightly', 'dumping') and the long-term and persistent nature of plastics ('microplastic', 'non-degradable') were the key reasons that respondents viewed plastics negatively. These results were similar to those found in a study by Dilkes-Hoffman et al. (2019) carried out on members of the public in which the same question was asked and led to positive answers such as; 'cost (cheap)', 'convenience' and 'usefulness' and negative responses including; 'waste/rubbish', 'pollution' and 'environment'. In this regard fishers are a reflection of

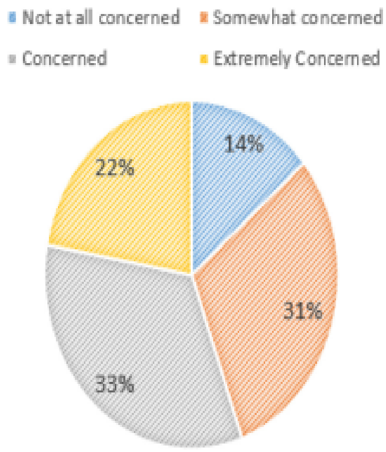

Fig. 7. Concern levels of Irish fishers on amount of plastic used in fishing equipment.

members of the public studied by Dilkes-Hoffman et al. (2019) in that they both recognise the usefulness of plastic material but also see it as something that can have damaging effects to the environment and human interaction with it. Additionally, 22% of respondents included both a positive and negative response to this question which reflects the duality of plastics in modern life and included responses such as; 'damaging, durable', 'cheap but not cheerful' and 'carcinogenic, convenient'. Further inductive analysis was carried out on the positive and negative phrases that fishers used to describe plastics. Amongst positive terms used by respondents, the majority, 67%, were focused on the general usability or functionality of plastic, 19% on the end of life and the remaining 14% categorised as general positive terms. General negative terms made up the majority of the negative responses (46%), followed by end of life issues/concerns (25%), the ubiquity of plastic/lack of alternatives (15%) and environmental issues making up the remaining 14% of phrases. The complete list of phrases mentioned along with frequency is displayed in Table 1.

Fishers were asked to rate their level of concern towards the amount of plastic currently used in fishing equipment with the highest proportion of respondents (33%) saying they were 'concerned' and only 14% were 'not at all concerned' in this regard (Fig. 7). The high levels of concern that Irish fishers feel towards the current levels of plastic use in fishing equipment coupled with the fact that the lack of alternatives for plastics was mentioned in their response to opinion on plastics it is likely that they would be open to switching to more sustainable replacement equipment as was noted in stakeholders in an Australian fishery (Wootton et al., 2022). However, this change could only occur if it was convenient, easy and cheap for recreational and commercial fishers and fishmongers (Wootton et al., 2022).

8.2.3. Fisher's interactions with litter and fishing plastics

The behaviour of respondents towards plastic use in fishing, their recycling habits and how they deal with litter in the environment was also examined. Respondents were asked; 'when conducting your fishing based activity how likely are you to remove waste material you notice from the natural environment?'. This was examined as comparative research on household waste management found that simply increasing awareness of environmental issues does not necessarily lead to effective practices (Skorstad and Bjørgvik, 2018). Responses fell onto a 5-point Likert scale that included the options; 'highly unlikely', 'unlikely', 'unsure', 'likely' and 'highly likely'. Respondents generally stated that they would be inclined to remove waste material they noticed from the natural environment (Fig. 8).

Table 1
Phrases associated with plastics.

Positive	Frequency	Negative	Frequency	Neutral	Frequency
convenient	10	litter	1	indestructible	1
durable	4	dumping	1	ubiquitous	1
good	1	unnecessary	2	packaging	1
cheap	10	unsightly	1	developing countries	1
easy cleaned	1	bad	1	mulroy Bay	1
essential	2	damaging	3	disposable	1
useful	1	not environmentally friendly	1	less plastic	1
easy	1	pollution	1	long lasting	2
handy	1	not enough alternatives	1	beach goers	1
positive	1	too prevalent	1	no bin	1
recycle	5	not needed	1		
reuse	1	ever lasting	1		
biodegradable	1	Persistent	1		
non-toxic	1	microplastic	1		
depend on it	1	non degradable	1		
perfect packaging	1	carcinogenic	1		
		single use	4		
		irresponsible	1		
		lazy	1		
		thoughtless	1		
		should be banned 1			
		lifetime	1		
		negative	3		
		no alternative	1		
		non recyclable	2		
		everywhere	1		
		disgusting	1		
		terrible	1		
		detrimental	1		
		harmful	1		
		needless	1		
		permanent	1		
		crap	1		
		unnecessary	1		
		not cheerful	1		
		painful	1		
		useless	1		
		poison	1		
		dirty	1		
		environment killer	1		
		rubbish	2		
		pollution	2		
		lifelong	1		
		bad	1		
		nonbiodegradable	1		
		longterm problem	1		
		not good	1		
		serious problem	1		
		damaging to environment	1		

Following this, the relationship was examined between the likelihood of waste removal and the fishing activity that respondents selected. Commercial fishers were the most 'likely' or 'highly likely' to remove litter they encounter while carrying out their activity (91%). This was followed by aquaculture (75%) and angling at 70% (Supplementary material Appendix 1, Fig. A4). The fact that commercial fishers, anglers and aquaculturists were, for the most part, likely or highly likely to remove waste material indicates they may have a level of care or understanding for the need to preserve the environment they enjoy a leisure activity in or livelihood from. Fishers may potentially be associating environmental care with direct economic benefit, which is parallelled with competent fisheries management (Asche et al., 2018). These results would indicate that while fishers are noticing waste in the environment they fish in both frequently and in medium–large volumes they are also likely to remove aquatic litter from the environment. This may be due to their direct experience from seeing litter frequently and understand the impacts it can have and take responsibility for its removal. A recent study on marine litter and fishers has shown that collecting waste material and storing it can be difficult on-board fishing vessels, especially smaller ones, due to issues such as; lacking capacity and lacking time and personnel for doing so (Olsen et al., 2020). However, interviews with fishers in the same study found that at least some fishers thought that 'whether litter is taken ashore is much about attitudes', as in, waste removal and collection from the environment will occur based on attitudes and is an independent factor from vessel size in general.

Marine plastic pollution remains a global threat with discarded fishing gear a major contributor (Kuczenski et al., 2022). Plastic is a major component of fishing gear including lines, netting, life jackets/waders, containers and lobster/crab pots, all of which can represent a disposal problem. In this study, respondents were asked to explain their reason for choosing certain plastic material for their selected fishing activity. Durability played a major role in the use of all plastic items, ranking first for all items save 'Lobster/crab pots' where cost played the greatest role in their use. Generally, recyclability and ease of disposal ranked medium-low (highest ranks of 3rd and 4th respectively out of 7 places) for reasons that fishers selected plastic items (Supplementary material Appendix 1, Fig. A5). This would indicate that end of life concerns do not play a major part in plastic item selection by Irish fishers, with their decisions primarily based on functional or economic incentives. Members of the Irish fishing community chose plastics for use in their activities despite the fact that they are broadly aware about the impacts plastic pollution can have and see litter frequently during

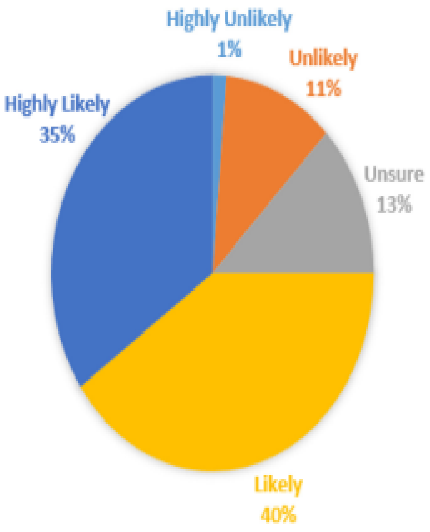

Fig. 8. Likelihood of removing waste from aquatic environment.

their activities and so awareness and knowledge is not really affecting their behaviour. However, this awareness and knowledge may mean they are open to alternative material for fishing plastics. Although not explicitly linked to this question on plastic use one individual offered up more information by selecting other and commenting; 'I would like to recycle in or disposal in a skip but the skipper directs me to dispose overboard'. This may indicate that human command may be a barrier to proper recycling habits on board Irish Sea vessels. Although recyclability did not rank in the top 2 reasons for the selection of any plastic products by Irish fishers in this study, the Irish public in general have a noted high willingness (89%) to use and buy plastic packaging and products that are made of 100% recycled plastic (EPA, 2022). The increased availability of fishing material made of recycled products could therefore lead to a more circular system for Irish fishing.

Fishers were also asked to define the disposal methods they used for the aforementioned 5 groups of plastic items (Supplementary material Appendix 1, Fig. A7). The following options were available for selection as answers; 'Pay for Disposal', 'Pay for Recycle', 'Repair/reuse', 'Domestic Waste Bin', 'Don't Know', 'I do not use these plastics' and 'Other'. For a true picture of how plastics are disposed of, the responses in the category; 'I do not use these plastics' were excluded for further analysis. Totalling the disposal methods of the 5 types of plastic items found that 72% of plastic items used were disposed of responsibly by fishers (Pay for disposal, pay for recycle, domestic waste bin), however it is unclear whether waste was segregated by fishers disposing of it. This is similar to a study carried out by Filho et al. (2021a) on the waste management of plastics used by members of the public which found the majority of respondents (74%) segregated plastic waste and disposed of it in a responsible manner. For a true picture of how unrecycled plastics are disposed of and to facilitate a greater understanding of waste management behaviour respondents could select the response 'other' and add a comment. While 'other' was selected 25 times for various types of plastic equipment just 44% of the 25 respondents elaborated on what they did with those plastics. Several responses mentioned recycling in some form e.g. 'We put ashore to a recycle (bin) on pier head' and 'Recycle centre', two more responses mentioned burning of the plastics they use. While most troublesome of all was the response: 'Discard back into the sea, by order of the skipper' indicating deliberate littering action of the natural environment. While this response is indeed worrying it must be noted that this is an isolated response to the survey amongst a plethora of more environmentally conscious respondents, it must also be noted the language of this response 'by order of the skipper', suggests an order a senior authority to litter the sea. In general, however, the majority of plastics used (72%) are disposed of responsibly indicating that fishers are taking responsibility for the plastics they use.

Fishers play a pivotal role in aquatic litter as they can easily add to it through littering of marine or freshwater environments (e.g. through dumping at sea, or the losing of gear) as well as being in a unique role of being able to retrieve marine or aquatic litter from otherwise remote locations and habitats. It must be remembered that barriers do exist to proper recycling in the fishing community be it logistical constraints or through to human behaviour. For this reason, having proper facilities and adequate infrastructure in place to make the disposal or recycling of items that can become marine litter as cheap and convenient as possible for this group of stakeholders would be of value.

In lieu of this, respondents were asked to indicate the barriers to recycling they face when trying to dispose of common fishing plastics (Fig. 9). Respondents could select one of; 'Lack of facilities', Don't know how to', 'Time consuming', 'Too expensive', 'Contamination (No. accepted)', or 'Other'. The options 'I do not use these plastics' and 'I recycle these plastics' were excluded to gain a true picture of the barriers faced. 'Lack of facilities' received the highest proportion of responses independent of the plastic equipment ranging from 33% to 55%. Netting was the plastic material with the highest proportion of responses in the 'too expensive' category with 17% of responses falling into this category. While 47% (n = 8) of respondents that selected 'other' wrote comments, two (n = 2) of these simply stated N/A. Two (n = 2) respondents stated there were no facilities to recycle fishing line. Two (n = 2) other responses indicated some form of professional waste disposal however they were unsure of the details stating; 'I do not recycle, it's (waste material) collected on the pier' and 'waste disposal company. One (n = 1) respondent stated that 'they kept old waders to use as patches' while finally 'Skipper says it's too time consuming to dispose of it even in a skip at the quay. None of the boats in Wicklow town use the skip, so all litter, like drinks bottles, sweet wrappers, cling film, old ropes get thrown overboard unfortunately.' The fact that household waste from the vessels operating out of Wicklow town may be ending up as marine litter is environmentally problematic particularly in plastic form given the myriad impacts it may have in this environment (UN Environment Programme, 2017). This statement is in direct contrast from those gathered via interview from fishers in Northern Norway (Olsen et al., 2020); 'It goes without saying we take care of our own trash'. The fact that this respondent acknowledges that there is a skip available in the quay for their waste and yet their waste is still thrown overboard while out at sea is in contrast to the heavily selected category of 'lack of facilities' for barriers to recycling, in that facilities are available and unused. While the main barrier to recycling different plastics were physical/logistical (lack of facilities) (n = 61, 43%), the 2nd most selected barrier was a more personal option of 'don't know how to' (n = 40, 28%) and so may indicate that members of the Irish fishing community could benefit from educational courses or material detailing how to recycle the equipment they use. Interviews with fishers and fishmongers in a South Australian fishery also suggested there were a lack of appropriate disposal locations, such as rubbish bins, throughout jetties and popular fishing spots which hindered proper waste disposal (Wootton et al., 2022).

In order to determine the views of recycling amongst Irish fishers, respondents were asked to list two words or phrases they associate with the topic. For ease of interpretation responses are represented in the form of a word cloud (Fig. 10). Responses were classed as either positive, negative or neutral. Some responses classed as neutral could also be described as non-descript in nature for example 'bin' or 'centre'. The largest portion (44%, n = 46) of terms were deemed as positive in nature with the descriptions 'good' and 'easy' recurring. The minority of terms (22%, n = 23) were classed as negative in nature.

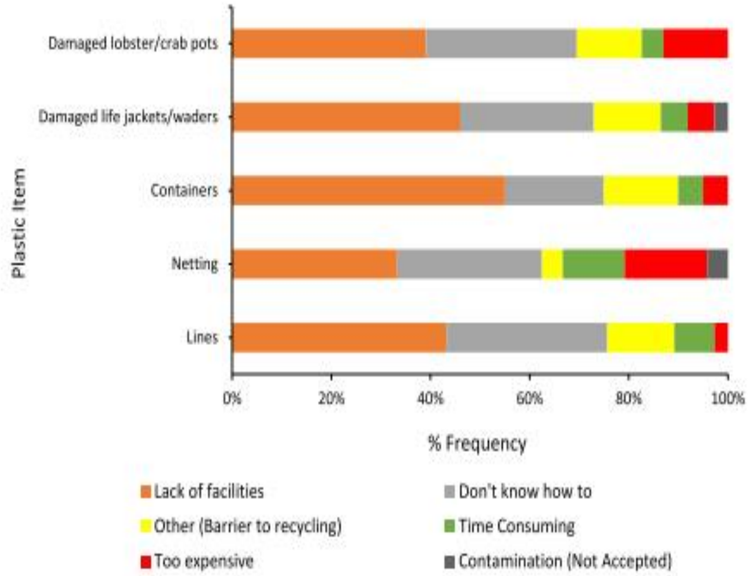

Fig. 9. Factors stopping the recycling of plastics used in fishing activities.

Fig. 10. Wordcloud displaying phrases fishers used to describe recycling.

The terms 'expensive' and 'inconvenient' were commonly used while issues around recycling facilities or receptacles were also mentioned. A significant proportion (35%, n = 36) of terms were deemed as neutral ('misunderstood') or ambiguous (e.g. 'hemp-based products',

Table 2
Phrases associated with recycling.

Positive	Frequency	Negative	Frequency	Neutral	Frequency
green environment	1	inconvenient	2	limited	2
worthwhile	1	too many unrecyclable plastics	1	sometimes, benefits not clear	1
very positive	1	not enough public recycling receptacles	1	bin	2
progressive	1	not enough	1	centre	1
		sceptical	1	why not get rid of the plastic in the first place?	1
good	4	expensive	2	responsible	1
healthy	1	inadequate	1	misunderstood	2
important	1	costly	1	hemp based products	1
good practise	1	misguided	1	reuse better	1
achievable	1	complicated	1	plastic	1
green	2	lack of facility	1	reuse	3
environmentally conscious	1	difficult	1	not moving quick enough	1
sensible	1	Not sure which bin to use if different materials in the one unit	1	reduce waste	1
positive solution	1	not cheap	2	clean before recycling	1
good practise	1	negative	1	do not know	2
needed	2	time consuming	2	pick up and bin	1
great idea	2	recycling centre refused to take my plastics	1	segregation	1
sustainability	1	not enforced	1	proper facilities	1
easy	4	confusing	1	more facilities	1
environmental friendly	2			reduce	1
very important	1			plastics	1
positive	1			rubbish	1
Try curb its use	1			see sense	1
extremely important	1			bring your rubbish home	1
renewable	1			almost unavoidable	1
reusable	1			please recycle	1
very good	1			hard plastic	1
environment saver	1			bottle banks	1
happy	1			recycled plastic	1
friendly	1			responsibility	1
helps	1				
essential	2				
cheerful	1				
good idea	1				
necessary	1				
right thing to do	1				

'segregation', 'plastics'). Inductive analysis of positive terms associated with recycling found that the majority were general positive comments (67%) followed by those emphasising the necessity of recycling (18%) and environmental terms made up the remaining responses (15%). With regard to the negative phrases associated with recycling the inconvenience and cost was the dominant theme noticed (52%), which was followed by the limitations of recycling/factors hindering more recycling (35%) and general negative phrases associated with recycling made up the remaining 13% of comments. The complete list of responses is displayed in Table 2.

9. Conclusion

This study provides novel insight into this understudied and unique group's attitudes. This research work should serve as a guideline for views that do exist within this niche group and is not intended to be representative of the whole Irish fishing community. This research presents a holistic first look at Irish fisher's awareness of a number of environmental issues relating to an emerging pollutant of environmental concern, i.e. microplastics. Amongst topics covered this study assessed; attitudes towards recycling and plastic, the perceived threat level of plastic pollution and the factors that hinder their recycling of plastic items they use in their fishing practises.

Fishers can be a point source of microplastics entering aquatic environments as they can be shed from discarded or lost fishing gear. Fishers are in a unique position for removing litter from remote regions and stop the production of secondary microplastics in the natural environment. Fishers can potentially be affected directly via microplastics impacting fish health or behaviour thus affecting recreational or economic activities. Furthermore, the reported presence of microplastic debris in seafood could lead to a consumer shift to different food produce in order to avoid this pollutant with fishmongers interviewed in a South Australian fishery noting that if microplastics were found in fish they sold to consumers it would wipe their business out (Wootton et al., 2022). While stakeholders in an Australian fishery assessed by Wootton et al. (2022) misperceived plastic pollution as less of an issue locally this was not the case for Irish fishers surveyed as part of this survey and they placed the foot of the blame of plastic pollution in aquatic environments at many groups themselves included. Fishers surveyed as part of this study were aware of microplastics and this may be due to a recent increase in news articles around this topic and their own interest in an environmental pollutant that been found in seafood around the world.

While a large amount of the ROI's fishing vessels are involved in the Fishing for Litter Initiative there is as yet no particularly innovative retrieval or preventative measures for marine litter in place in the ROI and further work is needed on this especially as it is an island nation. While the removal of litter from aquatic environments is crucial it also important to address issues such as poor waste management and unsustainable production and consumption habits which are the root causes of marine litter generation. In order for fishing to develop sustainably, fishers need the skills, knowledge and information to implement changes to more sustainable fishing gear and disposal methods. The importance of assessing this specialised group cannot be overlooked as fisher's relationship with microplastics and aquatic litter is a complicated one. Given that plastic production is expected to increase in the future and that current waste strategies are inadequate to deal with the production and consumption levels the ubiquitous presence of aquatic litter encountered by Irish fishers will continue.

Some limitations of this research are namely due to the smaller than anticipated sample size, however, as previously noted by Wyles et al. (2019) this cohort can be difficult to target with respect to surveying. Furthermore, this survey neglected asking fishers what could encourage them from moving away from plastic products in their recreational and commercial activities. This is an oversight on behalf of the research group, however, it can also stimulate further research questions for Irish fishing in the future and improvements in the sustainability of fishing are achieved in terms of biodegradable products and their efficacy.

The results of this study would suggest that improved and designated rather than general recycling facilities at access to fishing locations in combination with information on how to recycle fishing materials commonly used by Irish fishers could see a reduction in the amount of disused fishing equipment that may enter landfills and also the natural environment. Furthermore, fishers bemoaned a lack of alternatives to plastics available for them and were aware of the environmental issues associated with using plastics thus indicating an openness to replacement with more sustainable gear. Plastic was largely viewed as convenient by Irish fishers and this convenience would need to be replicated in replacement materials. Furthermore, any attempt to replace currently used and therefore potentially lost plastic fishing gear with more sustainable or biodegradable materials would have to ensure that these items are of equal or enhanced durability and quality in order to lead to a shift away from plastic fishing material as well as similarly priced as fishers stated they generally selected fishing plastics due to these factors. Further engagement with this crucial and understudied stakeholder group should be emphasised in order to develop effective policies to reduce litter presence and generation in aquatic environments.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.resenv.2023.100131.

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Survey Issued to stakeholders in Irish fishing

Survey for Participants in Fishing Activities

This study aims to assess the current level of understanding and opinions of stakeholders involved in fishing activities towards aquatic litter.

My name is Stephen Kneel and I am a PhD researcher in Dundalk Institute of Technology and I am hoping the results from this study will contribute towards my doctoral dissertation and possibly to the continued maintenance of the regions you conduct your fishing-based activities in (angling, aquaculture, fish-processing etc.).

You have been invited to take part in this survey as you are involved in fishing activities in some way in the Republic of Ireland. I am requesting you complete a short questionnaire 25 questions long which should only take between 5 and 10 minutes to complete as most of the questions can be answered by simply ticking a box. All information you provide will be private and confidential.

* Required

1. 1. I have read and understood the information provided and voluntarily agree to participate in this research project by giving my informed consent. *

Mark only one oval.

- Yes
- No

2. 2. Which of the following activities do you primarily take part in? (Please tick the most appropriate box) *

Mark only one oval.

- Angling
- Commercial Fishing
- Aquaculture
- Fish Processing
- Other:

3. 3. What county in the country do you primarily conduct your selected activities?

4. 4. Approximately how long have you been involved in your selected activity? *

Mark only one oval.

- 0 - 5 years
- 6 - 10 years
- 11 - 15 years
- 16 - 20 years
- 20+ years

5. 5. What age category do you fall under * *Mark only one oval.*

- 18 - 24
- 25 - 34
- 35 - 44
- 45 - 54
- 55 - 64
- 65 +
- Rather not say

6. 6. Are you? *

Mark only one oval.

- Male
- Female
- Non-Binary
- Prefer not to say
- Other:

7. 7. How frequently, as part of your selected activity (as selected in question 1), would you spend time out in the natural * aquatic environment (e.g. At Sea, fishing in rivers, tending aquaculture sites)?

Mark only one oval.

- Daily
- Every few days
- Weekly
- Fortnightly
- Monthly
- Never
- Other:

8. 8. When partaking in your selected fishing activity (as selected in question 1) how often would you notice the presence of * items that may be classed as waste material (e.g. plastic bags, water bottles, discarded fishing lines, etc)?

Mark only one oval.

- Never
- Rarely
- Occasionally
- Often
- Every time

9. 9. When partaking in your selected fishing activity (as selected in question 1) how many items would you normally notice * that may be classed as waste material (e.g. plastic bags, water bottles, discarded fishing lines, etc)?

Mark only one oval.

- 0 items
- 1 - 3 items
- 4 - 6 items
- 7 - 10 items
- 10+ items

10. 10. When conducting your fishing based activity (as selected in question 1), how likely are you to remove waste material * you notice from the natural environment?

Mark only one oval.

- Highly unlikely
- Unlikely
- Unsure
- Likely
- Highly likely

11. 11. How do you dispose of the following plastics used? Please tick all that apply. *

Mark only one oval per row.

	Pay for disposal	Pay for recycle	Repair / reuse	Domestic Waste Bin	Don't know	I do not use these plastics	Other
Lines (mono, uoro, braid, y-lines etc.)							
Netting							
Damaged life jackets / waders							
Containers (bait boxes, lure boxes etc.)							
Damaged lobster / crab pots							

12. 12. If you selected other for any of the above plastics, please specify.

13. 13. Please select which plastics you recycle and what barriers may prevent you from recycling the following fishing plastics. Please tick all that apply.

Mark only one oval per row.

	I do not use these plastics	I recycle these plastics	Too expensive	Don't know how to	Time consuming	Contamination (not accepted)	Lack of facilities	Other (Barrier to recycling)
Lines (mono, uoro, braid, y-lines etc.)	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Netting	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Containers (bait boxes, lure boxes etc.)	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Damaged life jackets / waders	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Damaged lobster / crab pots	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭

14. 14. If you selected other for any of the above plastics, please specify.

____

15. 15. Which factors influence your decision for using the following fishing plastics? Please tick all that apply. *

Check all that apply.

	Cost	Recycleability	Durability	Convenience	Ease of disposal	No alternative	I do not use these plastics	Other
Lines (mono, fluoro, braid lines etc.)	☐	☐	☐	☐	☐	☐	☐	☐
Netting	☐	☐	☐	☐	☐	☐	☐	☐
Life jackets / waders	☐	☐	☐	☐	☐	☐	☐	☐
Containers (bait boxes, lure boxes etc.)	☐	☐	☐	☐	☐	☐	☐	☐
Lobster / crab pots	☐	☐	☐	☐	☐	☐	☐	☐

16. 16. If you selected other for any of the above plastics, please specify.

____

17. 17. Over the time you have been involved in fishing activities have you noticed an increase in the use of plastics for your selected activity? *

Mark only one oval.

- ◯ Yes
- ◯ No
- ◯ Don't know

18. 18. How concerned are you about the amount of plastic used in fishing activities? *

Mark only one oval.

- Not at all concerned
- Somewhat concerned
- Concerned
- Extremely Concerned

19. 19. Where do you think plastic pollution in Rivers/Oceans is coming from? *

20. 20. Please list below two words/phrases you associate with plastics (either positive or negative). *

21. 21. Please list below two words/phrases you associate with the word 'recycling' (either positive or negative). *

22. 22. Have you heard of the term microplastic? *

Mark only one oval.

- Yes
- No
- Don't know

23. 23. If you indicated yes to the previous question please give your understanding of the term 'microplastic'.

24. 24. Please indicate to what extent you are aware of the following: *

Mark only one oval per row.

	Not aware	Somewhat aware	Aware	Very aware
Plastic pollution in freshwater environments (Rivers/lakes)	⬭	⬭	⬭	⬭
Microplastic pollution in freshwater environments (Rivers/lakes)	⬭	⬭	⬭	⬭
Plastic pollution in the oceans	⬭	⬭	⬭	⬭
Plastic pollution interacting with marine or riverine life (fish, insects, birds etc)	⬭	⬭	⬭	⬭
Microplastic pollution in the oceans	⬭	⬭	⬭	⬭
Microplastic pollution interacting with marine or riverine life (fish, insects, birds etc)	⬭	⬭	⬭	⬭
Plastic pollution on land	⬭	⬭	⬭	⬭

Microplastic pollution on land	⬭	⬭	⬭	⬭

25. 25. How serious a threat do you think plastic pollution poses for each of the following: *

Mark only one oval per row.

	Not at all serious	Somewhat serious	Serious	Very serious
Irish shing industry	⬭	⬭	⬭	⬭
Freshwater environments (lakes and rivers)	⬭	⬭	⬭	⬭
Oceans	⬭	⬭	⬭	⬭
Marine and freshwater wildlife (sh, seabirds etc)	⬭	⬭	⬭	⬭
Terrestrial Environments (land)	⬭	⬭	⬭	⬭
Humans	⬭	⬭	⬭	⬭

Google Forms

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