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University of Southampton

Faculty of Environmental Life Sciences

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Ecosystem Level Impacts Of Plastic Pollution

by

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University of Southampton

Abstract

Faculty of Environmental Life Sciences

Ocean and Earth Science

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The plastic lifecycle intersects with critical global challenges, including climate change, biodiversity loss, and pollution across soil, air, and water systems. Despite extensive research into the sources, pathways, distributions, and impacts of plastic pollution, particularly within marine environments, significant knowledge gaps persist. Regions experiencing severe mismanaged waste and plastic leakage, often associated with ecological sensitive habitats, remain particularly under-studied. Additionally, data fragmentation and methodological inconsistencies impede comparative analyses, while limited technical and financial capacity constrains efforts to monitor pollutants and assess their effects on human and environmental health. This study addresses some of these gaps by identifying accessible methods for quantifying marine plastic pollution and assessing potential ecosystem-level impacts across lower and higher trophic level communities. Key objectives included reviewing current knowledge on ecosystem-level effects related to the plastic life cycle, evaluating existing monitoring initiatives, and identifying policy-orientated research gaps from an academic perspective. Considering fieldwork limitations due to COVID-19, the study investigated existing and innovative, cost-effective techniques to promote a comprehensive and inclusive approach towards monitoring marine anthropogenic pollution. First, an experiment was designed to examine the effects of surface macroplastic loads and associated communities on ambient lower trophic level communities. This resulted in a simple and reproducible method for quantifying the potential impact of plastic-associated communities on local ecosystems and productivity. Second, a global isotope dataset was utilised to evaluate the feasibility of detecting pollution loading, specifically anthropogenic nitrogen, in the physiology of higher trophic level organisms. This represents the first application of this approach, supporting further investigation into the use of global mesopredatory elasmobranchs as indicators of anthropogenic pollution. Finally, a rapid marine litter assessment was conducted around the UK to quantify beach litter, comparing outputs from two widely applied monitoring approaches. While findings demonstrated strong similarities, notable site-level differences were observed.

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List of accompanying materials

Alongside and in alignment with this research are two published reports:

Maes, T., Preston-Whyte, F., Lavelle, S., Gomiero, A., Booth, A.M., Belzunce-Segarra, M.J., Bellas, J., Brooks, S., Bakir, A., Devriese, L.I. and Pham, C.K., 2023. A recipe for plastic: Expert insights on plastic additives in the marine environment. *Marine pollution bulletin*, 196, p.115633. doi.org/10.1016/j.marpolbul.2023.115633.

Lavelle, S., Preston-Whyte, F., Lamin, P.A., Sankoh, S.K., Timbo, I. and Maes, T., 2024. Troubled water: Tracing the plastic tide on Sierra Leone's beaches. *Cambridge Prisms: Plastics*, 2, p.e28. doi:10.1017/plc.2024.27.

Two additional papers are in final review and will be published in the coming weeks. One reports a survey conducted in Indonesia concerning microplastic in seafood, while another assessed proficiency of seafloor litter monitoring.

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Photos: Taken from site visits in Sri Lanka and Sierra Leone.

Research Thesis: Declaration of Authorship

Stephanie Lavelle

Title of thesis: Ecosystem Level Impacts of Plastic Pollution

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. None of this work has been published before submission except the Policy Brief (appendix IIII)

Signature:

Date: 29/05/2025

Chapter 1: An introduction to the global challenge and research landscape of plastic pollution

1.1 The growing problem of plastic production

The global plastic market is characterised by robust production, with over 460 million metric tonnes (Mt) produced annually and over \$200 billion (USD) invested in petrochemical production over the last decade (OECD, 2022; UNEP, 2021a). Plastic is a versatile, affordable, durable and useful material used across most industries and consumer products (Geyer et al., 2017). As a result, projections suggest that plastic production could triple by 2050 (Borrelle et al., 2020a; Geyer, 2020), particularly in response to growing populations and economies across Africa and Asia (Lebreton and Andrady, 2019a).

Despite its many benefits, the scale of production, incorporation of chemical additives, lack of circularity, and mismanagement of waste are causing serious environmental impacts (Tsakona et al., 2021; UNEP, 2021a; Wagner et al., 2024). Most plastics are derived from fossil fuel-based sources and thus represent a key driver of climate change through extraction, production processes, consumption and waste management practises (Ford et al., 2022). Currently, production processes consume approximately 4-8% of global oil production for material feedstock and energy, a figure expected to increase to 20% by 2050 (Cabernard et al., 2022). Additionally, over 16,000 intentionally added chemicals have been identified in plastic production, fewer than half of which have been studied. Yet, around a quarter of those that have been studied have been classified as hazardous and are of concern to human and environmental health (UNEP and BRS Secretariat, 2023; Wagner et al., 2024). Furthermore, thousands of unintentionally added chemicals and substances are present in plastics due to manufacturing processes, contamination and degradation (Wiesinger et al., 2021).

The plastics economy follows a linear and resource-inefficient model (Borrelle et al., 2020a). Addressing this issue and its diverse repercussions necessitates a fundamental shift towards sustainable consumption and production practises, promoting the safe, efficient, and circular use of plastic within the economy (Vidal et al., 2024). It is

imperative to recognise that certain plastic applications may not be conducive to circularity and may require redesign, substitution or elimination (Jambeck and Walker-Franklin, 2023). Scientific evidence underscores that transitioning towards a comprehensive circular plastics economy could significantly mitigate plastic pollution across its lifecycle (Lau et al., 2020).

1.2 Plastic pollution sources, pathways and drivers

Assessing the mass balance of plastics and quantifying pollution loading across different environmental compartments is crucial for investigating potential ecosystem-level impacts (Harris et al., 2023). However, quantifying materials of different sizes, buoyancy, and composition that are constantly moving through dynamic systems remains inherently complex (Li et al., 2021). Despite challenges, it is widely accepted that most plastic waste is retained on land, while the majority of marine litter originates from land-based sources (Galgani et al., 2015; Meijer et al., 2021). Upon entering coastal and marine environments, buoyant plastics can continuously move between their terrestrial and aquatic systems, floating on the surface or within the water column (Harris et al., 2023). Eventually, they may become buried along coastlines or settle on the seafloor, which serves as the ultimate sink for marine plastics (Martin et al., 2022).

Plastic leakage into both terrestrial and aquatic environments occurs at various stages of the plastic life cycle. Mismanaged plastic waste is the primary source of macroplastic leakage, accounting for over 80% of plastic entering the environment, with littering contributing around 5% (OECD, 2023). Estimates of plastic waste generation and leakage rates suggest that between 5-20 million Mt escape into aquatic environments annually (Borrelle et al., 2020b; Jambeck et al., 2015a), a figure that could rise to over 50 million Mt by 2030 (Borrelle et al., 2020a). Models and studies further suggest that rivers are significant drivers of plastic waste into oceans, with discharge estimates ranging between 0.8 and 2.7 million Mt annually and small urban rivers ranking among the most significant contributors (Al-Zawaidah et al., 2021; Meijer et al., 2021).

It is well-established that population and gross domestic product (GDP) are significant drivers of municipal solid waste (MSW) generation rates and subsequent mismanaged

plastic waste (Jambeck, 2015; Lebreton and Andrady, 2019; Meijer, 2021; Nyberg, 2023). The relationship between GDP per capita and MSW generation rates is positively correlated, meaning that as GDP per capita increases, so does the rate of MSW generation (Lebreton and Andrady, 2019). Conversely, GDP is negatively correlated with the proportion of waste that is mismanaged, indicating that nations with higher GDP tend to have lower levels of mismanaged waste (Lebreton and Andrady, 2019). Additionally, a significant global challenge is that plastic waste reportedly shipped for recycling is often exported from high- GDP countries to lower GDP-nations with weaker regulatory enforcement, where it frequently becomes mismanaged and leaks into the environment (Gündoğdu and Walker, 2021).

Modelling studies indicate that regions in Africa and Asia, which contain crucial habitats and rich biodiversity, exhibit the highest rates of plastic leakage (Jambeck et al., 2018; Lebreton and Andrady, 2019a; Meijer et al., 2021). However, data on MSW generation rates, plastic waste generation, and waste management practices remain outdated or insufficient across many regions (Akindele and Alimba, 2021; Kapinga and Chung, 2020). Direct methods for monitoring methods primarily focus on tracking plastic after it has escaped production, consumption, and waste streams. Among these, beach litter monitoring is one of the most commonly used approaches due to its cost-effectiveness. However, as with many plastic monitoring approaches, the methods employed vary widely and studies are often spatially and temporally fragmented (Fanini et al., 2021).

1.4 Plastic pollution impacts

The current plastic life cycle is a key driver of climate change, pollution, and biodiversity loss, pushing the planet beyond critical boundaries from which recovery may not be possible (Almroth et al., 2022; Larsen and Tararas, 2024). The challenge in monitoring pollutants, including plastics and their associated additives, lies in their wide variation in bioavailability and impact, exhibiting a range of direct, persistent, sporadic, bioaccumulative, and indirect effects (Geyer et al., 2000; Matthies et al., 2016). Their sources may be point-based or diffuse, continuous or sporadic, further complicating monitoring efforts (Yuan et al., 2007).

Once released into aquatic environments, plastics can disrupt food web dynamics through ingestion, entanglement, and smothering (Li et al., 2021). Additionally, plastics serve as novel substrates that can alter habitat structures by providing surfaces for the colonisation of microorganisms and lower trophic level species. This colonisation can impact plankton communities, nutrient fluxes, and overall ecosystem functioning (Amaral-Zettler et al., 2020; Li et al., 2021). Floating debris may act as shelter for juvenile fishes, potentially influencing predator-prey interactions, foraging behaviour, growth, and survival.

The vulnerability of marine populations to plastic pollution depends on exposure, species sensitivity, and population resilience (Kühn and van Franeker, 2020). However, current research has primarily addressed plastic entanglement and ingestion, particularly microplastics, with studies focusing on a limited range of species (Kühn and van Franeker, 2020; Provencher et al., 2019). Although biomonitoring efforts have surveyed seabirds, sea turtles, marine mammals, fish, and invertebrates (Bonanno and Orlando-Bonaca, 2018; Kühn and van Franeker, 2020; Savoca et al., 2022), significant gaps remain regarding keystone species, spatial-temporal variations, and ecosystem-specific responses (Provencher et al., 2019). Critical aspects such as abundance, distribution, ingestion rates, trophic transfer, and biomagnification are still poorly characterised (Clark et al., 2023; Provencher et al., 2019; Savoca et al., 2022).

Furthermore, the absence of universal definitions and standardised quantification methods impedes comparisons across studies and limits the development of reliable indicators to assess plastic pollution throughout its lifecycle (Kühn and van Franeker, 2020; Savoca et al., 2022). Laboratory studies to date have largely focused on microplastics, single chemicals, or single species, predominantly invertebrates and phytoplankton, leaving a broader range of trophic interactions insufficiently explored (Maes et al., 2023). Additionally, a lack of transparency and unclear responsibilities for monitoring plastic production, including hazardous additives, creates significant data gaps, hampering our understanding of their cumulative impacts on ecosystems and human health (Groh et al., 2019a; Wiesinger et al., 2021).

1.5 Monitoring and reporting

Achieving a reduction in plastic pollution will ultimately necessitate comprehensive and harmonised monitoring efforts that track plastic across its lifecycle, linking production, trade, consumption, waste management, and environmental leakage. A range of global and regional initiatives are actively working to harmonise monitoring approaches and establish robust material flow accounts, as well as indicators, to track plastic flows and the impact of mitigation efforts (Cottom et al., 2024; GESAMP, 2019; Kawecki et al., 2018; Maximenko et al., 2021; UNEP, 2023a). Monitoring efforts and established indicators have predominantly focused on waste management and end-of-life plastic flows and metrics. However, there has been a growing effort to capture upstream production and trade dynamics (Table 1.1) (Barrowclough et al., 2020; Barrowclough and Birkbeck, 2022; Kawecki et al., 2018; UNEP, 2021b). For example, although considered to be an underestimate due to hidden plastics within monitored trade streams, trade data regarding plastics has become more accessible through various tools and data platforms, such as the UNCTAD trade data hub (Barrowclough et al., 2020).

Table 1.1: Recommended metrics from ongoing efforts to monitor plastic in the economy and in the environment at a global level (Foschi and Bonoli, 2019; UNEP, 2021a).

Life cycle stage	Metric	Source of data or indicator
Production & trade	Domestic plastic production	Industry reports and Customs Procedure Codes (CPC)
	Total plastic exports and imports, including plastic waste	Trade and customs ministries
	Total plastic production, per polymer type and application	Data available from industry or can be estimated using trade and production codes
Waste generation	Total plastic waste generated	New Plastics Economy Global Commitment
Waste management	Proportion of MSW collected and managed out of total generated, by city	UN SDG indicator 11.6.1
	National recycling rate, tons recycled	UN SDG indicator 12.5.1
	Total plastic waste recycled	New Plastics Economy Global Commitment
	Amount of recycled plastic going into new products	EU Single Use Plastics Directive
Emissions	Plastic debris density	UN SDG indicator 14.1.1b

International efforts that are advancing plastic monitoring and reporting across the lifecycle include the New Plastics Economy Global Commitment, with over 500 signatories committed to actions spanning the plastic product lifecycle (Defruyt, 2019). Additionally, various initiatives are developing plastics action plans, inventories, and indexes to promote effective management and mitigation (Diana et al., 2022; Smol, 2023). The World Trade Organisation's dialogue on plastic pollution aims to enhance transparency in trade flows, an area where data remain fragmented and inconsistent (Raubenheimer and McIlgorm, 2018). Furthermore, ongoing efforts to reform the Harmonised Commodity Description and Coding System (HS) seek to improve the classification and tracking of plastic goods in trade statistics (TESS et al., 2022).

Regarding waste generation monitoring and reporting, the United Nations (UN) Habitat's Waste Wise Cities tool has facilitated the collection of waste-related data from over 170 cities worldwide, contributing to AI-driven modelling of plastic generation, management, and emissions (Cottom et al., 2024). In addition, the Basel Convention on transboundary waste, currently the only global, legally binding instrument specifically addressing plastic waste, provides guidance for managing plastic waste and a toolkit to support the development of a plastic waste inventories (Bank, 2022).

The UN Sustainable Development Goal (SDG) 14, Life Below Water, is the only SDG explicitly addressing plastics, emphasising the need to “prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution” by 2025 (UNEP, 2021b). This target encourages the alignment of monitoring efforts with these critical indicators where feasible. Within this framework, national-level indicators include plastic debris densities, chlorophyll-a concentrations, total nitrogen, carbon, phosphorous, and silica. National statistical offices compiling this data could subsequently provide essential baseline information to assess ecosystem health, identify pollution hotspots, and support regional and global studies that inform policy decisions.

Global policies have also supported the use of indicator species to assess the impact of plastics on marine ecosystems, aligning with other monitoring and mitigation efforts (Zettler et al., 2013). Notably, SDG 14.1.1b includes a supplementary bioindicator of plastic ingested by biota (UNEP, 2021b). Additionally, the EU Water Framework

Directive (WFD: 2000/60/EC) and the Marine Strategy Framework Directive (MSFD: 2008/56/EC) require Good Environmental Status (GES) to be achieved, considering 11 key descriptors (Murillas-Maza et al., 2020). Of these, Descriptor 10 specifies that indicators should be developed to help achieve a level of marine litter that does not adversely affect relevant species, suggesting a supplementary stomach content analyses indicator (Rombouts et al., 2013). While this criterion has not yet been fully developed, it encourages regional and subregional cooperation to establish a list of species from key taxonomic groups (birds, mammals, reptiles, fish and invertebrates), define threshold values, and evaluate the impact of litter on marine organisms. The Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) guidelines further emphasise ongoing efforts to establish criteria for selecting bioindicator species and developing effective monitoring programs (GESAMP, 2019).

Despite numerous global initiatives aimed at addressing plastic pollution, significant challenges remain in establishing a coordinated and effective monitoring framework. Limited resources, inconsistencies, and fragmented data-sharing and reporting mechanisms, from municipal to national, regional, and global levels, continue to hinder efforts to track plastic pollution comprehensively. Additionally, while much research has focused on microplastics, the potential ecosystem-level impacts of macroplastics remains relatively understudied, particularly regarding their influence on keystone species and sensitive ecosystems that frequently experience high plastic loads. These gaps, along with the need for integrated, standardised, and scalable approaches to monitoring plastic pollution, underpin the rationale for this study. This research aims to identify accessible and effective methods for quantifying marine plastic pollution and to explore its potential impacts across different trophic levels, contributing to a more comprehensive understanding of macroplastic pollution dynamics and its ecological consequences.

1.6 Aims and objectives of study

There is a clear lack of harmonised indicators to understand the abundance, distribution and effects of plastic pollution on ecosystem structure and function. Some of the main barriers to enhancing our knowledge of the risks and impacts of plastic pollution stem from the complex diversity of plastic materials, the dynamic nature of

their distribution, and the limited resources available to research and address this global waste crisis.

The overall aim of this study was to identify easily employable methods for quantifying marine plastic pollution and assessing potential ecosystem-level impacts on lower and higher trophic level communities. The specific objectives were:

- Design an experiment to study the potential effects of surface macroplastic load and associated communities on food web dynamics at the base of the marine ecosystem.
- Utilise a global isotope dataset to assess mesopredatory sharks as a potential bioindicator for anthropogenic pollution, due to their relatively high-trophic level and ability to reflect localised food web biochemistry.
- Conduct a rapid marine litter assessment around UK beaches, comparing two commonly applied transect methodologies.

A multi-method approach was employed, combining literature reviews, analysis of existing data, innovative laboratory analysis, and field assessments of widely used monitoring approaches.

Chapter 2: Exploring the impact of floating macroplastics on lower trophic level communities in aquatic ecosystems: A Cost-Effective Investigation

2.1 Abstract

Floating macroplastic debris presents a growing threat to river ecosystems, prompting increased research attention. This chapter introduces riverine litter research, examining current volumes and distributions, while investigating potential impacts on lower trophic level organisms. Acknowledging existing data gaps and research limitations, especially in resource-limited environments, a simple and cost-effective experimental design was explored to investigate the influence of floating macro(plastic) debris and associated biofilms on ambient communities at the base of the food web. Preliminary findings from a trial microcosm experiment, which considered variations in plastic type, load, and exposure, revealed the potential to identify and characterise significant changes in productivity and species composition associated with the presence of floating debris that had been exposed to the marine environment. This conceptual experimental design could be applied to further investigate the diversity and interactions of communities associated with floating debris. To further explore the potential impact of plastics on the associated biofilm community, other non-plastics materials should be considered in future studies. The prospect for applying this experimental setup in remote regions with limited resources is discussed, opening opportunities for broader inclusivity in research efforts. Future refinement and expansion of this methodology is also considered, proposing avenues for further research.

2.2 Introduction

2.2.1 Plastic pollution in rivers

Escalating volumes of plastic waste entering aquatic ecosystems is an inevitable consequence of continually increasing mismanaged plastic production (Jambeck et al., 2015b; UNEP, 2021a). Current plastic production estimates surpass 400,000 million metric tonnes (Mt) annually, roughly equivalent to the biomass of the human population in new plastic material every year (Geyer, 2020; Lebreton and Andrady, 2019b; Tsakona et al., 2021). If growth trends persist, this will reach over 1 billion Mt by 2050 (Geyer, 2020). Despite the predominance of eight polymers in primary plastics, the addition of diverse chemical substances for functionality, aesthetics, or as bioproducts and contaminants result in a wide array of materials, many of which are short-lived and challenging to manage at the end of their lifecycle (Geyer, 2020; UNEP 2021).

Tracking plastic pathways beyond the production phase is an increasingly complex task along a predominantly linear plastic lifecycle. It is widely acknowledged that approximately 10% of plastics are sent for recycling globally, around 14% are incinerated, with the remaining 76% ultimately ending up in landfills, dump sites, or the environment (Geyer, 2020). Rivers are vital conduits linking land-based sources and the marine environment. Subsequently, they have become significant repositories of plastic debris, raising serious concerns for environmental integrity and human well-being (UNEP, 2021; UNEP and BRS Secretariat, 2023). The gravity of this issue is underscored by the volumes of plastic waste projected to be flowing through river and watershed systems (Harris et al., 2021b; Lebreton and Andrady, 2019b; Nyberg et al., 2023).

In the past decade, studies exploring plastic pollution in rivers have revealed some of the complexities associated with this environmental challenge (Gasperi et al., 2014; Lechner et al., 2014; Moore et al., 2011). For instance, actively meandering rivers function as extended reservoirs for plastic debris, with significant loads becoming embedded in sediments, further complicating monitoring and clean-up efforts (Nyberg et al., 2023; van Emmerik et al., 2022; van Emmerik and Schwarz, 2020). Plastic accumulation in rivers is known to be exacerbated by heavy rainfall events and has also been identified to extend beyond major channels into critical areas such as riverbanks,

mangroves, seagrass beds and river mouths (Okuku et al., 2022; Ryan and Perold, 2021; van Emmerik and Schwarz, 2020).

2.2.2 Quantifying the global extent of riverine plastic pollution

Estimating the extent of plastic release into aquatic environments at a global scale poses significant challenges. Based on plastic waste generation and leakage estimates, models have predicted between 5-23 million Mt of plastic pollution enter aquatic systems annually from land-based sources (Borrelle et al., 2020c; Jambeck et al., 2015b). Models generally agree that countries with large coastlines, urban centres and poor waste infrastructure represent some of the largest leakage points (Jambeck et al., 2015b; Lebreton and Andrady, 2019b; Meijer et al., 2021). Furthermore, Lau (2020) projects that the amount of plastic entering the marine environment will increase 2.6-fold until 2040 under a business-as-usual scenario.

Modelling techniques, calibrated with field data, have provided valuable baseline estimates of global plastic waste fluxes from rivers (Harris et al., 2021b; Meijer et al., 2021; Nyberg et al., 2023). One of the most recent endeavours to quantify the distribution of global plastic emissions estimated that over 1,000 rivers account for more than 80% of global land-based plastic emissions, with global influx volumes ranging from 0.8 to 2.7 million Mt per year (Meijer et al., 2021). Models also highlight the disproportionate contributions of smaller rivers in proximity to urban areas, particularly in Africa and Asia, to the global plastic pollution crisis (Lebreton et al., 2017; Meijer et al., 2021; Schmidt et al., 2017). However, despite being highlighted as significant contributors, such regions remain understudied, hindering a comprehensive understanding of the global plastic pollution landscape (Akenji et al., 2020; Akindele and Alimba, 2021; Lebreton et al., 2017; Meijer et al., 2021).

It is important to note that disparities persist in models, as they often generalise mismanaged plastic waste percentages, leading to inaccuracies in emission calculations, especially in low-income countries where there is limited or non-existent data (Lam et al., 2020; Mai et al., 2019). Alternative models, considering human development index and socioeconomic factors, offer more conservative estimates of plastic litter loads, further highlighting the need for a more comprehensive approach to

estimate global plastic emissions accurately (Mai et al., 2020). Additionally, field studies, vital for validating models, have focused on estimating plastic emissions in marine, estuarine, and to a lesser extent, freshwater environments (Bruge et al., 2018; Eriksen et al., 2014a; Lahens et al., 2018; Morritt et al., 2014).

Studies that have focused on riverine plastic pollution have primarily been conducted over short-timescales, concentrating on high-income countries and microplastics (Blettler et al., 2018; Calcar and Emmerik, 2019). Localised investigations have unveiled some of the highest reported plastic densities from specific studies of waterways across Asia and Africa, each providing unique insights into the sources and extent of macroplastic pollution (Ebere et al., 2019; Emmerik et al., 2019; Lam et al., 2020; van Emmerik and Schwarz, 2020). Some of the most prevalent litter items identified in these studies include single-use food and drinks packaging, however, quantities are difficult to compare due to lack of aligned research methodologies (Table 2.1). Thus, field data limitations and the absence of universal methods make it challenging to provide an accurate breakdown of national or regional river flux estimates.

Table 2.1: Summary of reported quantification across a selection of riverine debris studies from Africa and Asia.

Location	Rivers sampled	Quantification of riverine debris	Types of litter	Author
South Eastern Nigeria	5	3,478 macrodebris items (>5 cm) per m ²	Plastics (59%), metal (10%), cloth (7%), paper/cardboard (7%), rubber (7%), glass/ceramics (5%), medical and agro-based waste (3%), and wood (2%) - of count data	Ebere et al., 2019
Akwa Ibom, South Nigeria	3	0.9 to 2 tonnes per km ²	Suspended debris showed debris comprised of plastic, nylon, can, foil and "others"	Babatunde et al., 2018
Jakarta, Indonesia	5	Mean hourly plastic flux between 3 to 20 × 10 ³ items h ⁻¹ ; 2.1 × 10 ³ tonnes of plastic waste exported a year	Plastic between 37-54% mass content; Most of the plastic was identified as either soft polyolefins (38% mass) or multilayer (35% mass)	Emmerik et al., 2019
Pearl River Estuary, China	1	Macroplastics (>5 mm): 0.110 ± 0.039 items/m ³ ; micropastics (0.35-0.5 mm): 2.376 ± 0.700 items/m ³	This study highlights the Pearl River Estuary is the least polluted in comparison to other estuaries studied in China by up to 1,200 times	Lam et al., 2020
Saigon River, Vietnam	1	Plastic debris entering the river estimated from 0.96 to 19.91 g inhabitant ⁻¹ d ⁻¹ ; fibres and fragments across 4 canal systems were respectively 172,000 to 519,000 items m ⁻³ and 10 to 223 items m ⁻³	Macroplastics and fragments were mainly made of polyethylene and polypropylene while the anthropogenic fibers were mainly made of polyester	Lahens et al., 2018

The journey of plastic debris in aquatic environments, from its sources to sinks, is a multifaceted process influenced by specific properties, including density, shape, and environmental factors (Schwarz et al., 2019; van Emmerik and Schwarz, 2020). Notably, despite the heavy density of polyethylene terephthalate (PET), the shape of plastic bottles has made them one of the most abundant materials identified in riverine and coastal studies, alongside other forms of plastic packaging and films (Calcar and Emmerik, 2019; Ebere et al., 2019; Lavelle et al., 2024). While global modelling studies have provided valuable insights regarding the abundance and distribution of plastic debris, they often lack information such as polymer type, item shape and potential residence times, necessitating more empirical data to accurately assess plastic leakage and degradation in the environment (Jambeck et al., 2015b; Mai et al., 2020; Meijer et al., 2021). It should also be noted that there is a substantial lack of relevant production, consumption and waste generation data due to lack of transparency and data collection from industry and municipalities (Lebreton and Andrady, 2019b; Wiesinger et al., 2021). Subsequently, studies are weighted in an attempt to estimate marine pollution and microplastics (Nielsen et al., 2020).

Studies of floating plastic in the marine environment have predominantly focused on the smaller size fractions accumulating in oceanic gyres. Estimating floating microplastics (93-236,000 Mt) only account for a small fraction of the total plastic waste released (Eriksen et al., 2014a; Sebille et al., 2015), although, surface trawl observations could be significantly underestimated (Lebreton et al., 2018). There are still large data gaps, but most marine plastic debris is thought to remain suspended in the water column, eventually finding its ultimate sink on the seafloor, unless ingested or washed back ashore (Canals et al., 2021; Harris et al., 2023). Some studies suggest that, despite an increase of global plastic production, floating microplastics have not been measured to increase at a similar rate (Andrady, 2017; Eriksen et al., 2014a; Schwarz et al., 2019; Sebille et al., 2015). This highlights that the pathways and fate of plastic in the ocean is poorly understood and parameterisation of residence and degradation times is lacking (Wayman and Niemann, 2021). Overall, the diverse methodologies and objectives across studies and environmental compartments have resulted in often incomparable results, making a comprehensive assessment to guide effective mitigation strategies and policy formulation challenging (van Emmerik and Schwarz, 2020).

2.2.3 Ecosystem effects of floating macroplastic in aquatic environments

The widespread presence of macroplastics in aquatic systems poses significant challenges to both the environment and human health (UNEP, 2021a; UNEP and BRS Secretariat, 2023). While much research has focused on the direct impacts of macroplastics, such as ingestion and entanglement affecting specific species, less attention has been given to the broader ecological effects of floating macro debris.

Depending on the volume of the debris, it poses the potential to alter the physical characteristics of aquatic environments, such as light penetration and nutrient availability (Mincer et al., 2019; Nelson et al., 2021). Additionally, floating macro debris provides a unique substrate for marine organisms and facilitates biofouling processes (Ogonowski et al., 2018). Biofouling, defined as the colonisation of surfaces by microorganisms, phytoplankton, zooplankton, and larger marine invertebrates, was first documented on floating plastics over 50 years ago (Carpenter and Smith, 1972). Despite recent advances in biofouling research, particularly over the past decade, the ecosystem-wide effects on local biodiversity and community dynamics remain poorly understood (Ogonowski et al., 2018).

The colonisation process on a surface can begin within hours, starting with biofilm formation as microbial organisms develop an extracellular matrix (Caruso, 2020). This creates a distinct microbiome known as the 'plastisphere' when associated with plastic debris (Wright et al., 2020; Zettler et al., 2013), which alters plastic properties such as buoyancy and roughness (Cheng et al., 2021), and can affect nutrient cycling and ecosystem productivity (Mincer et al., 2019). The plastisphere may also act as a vector for harmful algae, pathogens, and invasive species, posing threats to vulnerable ecosystems (Barnes et al., 2009; Jeong et al., 2023; Kirstein et al., 2019; Zettler et al., 2013). Limited research has examined the role of eukaryotes within the plastisphere, highlighting the need to investigate how plankton communities respond to the potentially increasing presence of plastic surfaces (Schwarz et al., 2019; van Emmerik and Schwarz, 2020).

Plastisphere composition varies based on factors such as plastic hydrophobicity (Ogonowski et al., 2018) and the adhesive traits of colonisers (Kirstein, 2018). Some

studies suggest a distinct "core" plastisphere community (Amaral-Zettler et al., 2020), identifying communities to be different from surrounding and non-plastic substrate communities, particularly in the early stages of colonisation (Kettner et al., 2019; Ogonowski et al., 2018; Rech et al., 2018). Some studies have also found biofilm communities associated with certain polymers (Kirstein et al., 2019; Zettler et al., 2013), but over time, the influence of location, seasonal changes, and substrate type further complicates the characterisation of plastisphere communities (Lobelle and Cunliffe, 2011; Oberbeckmann et al., 2014; Pauli et al., 2017). A review of current research concluded there is not sufficient evidence to show that the plastisphere community differs from communities on other surfaces, particularly in the later stages of colonisation (Wright et al., 2020). Despite some progress, gaps remain in understanding the functional roles of plastisphere communities and their ecological impacts (Wright et al., 2020).

Most research concerning the plastisphere has focused on microplastic colonisation studies in the open ocean, with few studies addressing river systems in regions with high plastic pollution (Kettner et al., 2019; van Emmerik and Schwarz, 2020). Common methods, such as microscopy and molecular dyes, have provided insights into species richness, yet advanced DNA sequencing remains underutilised due to high costs and technical demands (Amaral-Zettler et al., 2020). Understanding how floating (plastic) debris affects lower trophic levels and carbon cycling is crucial, especially in low-income regions where data is scarce and plastic pollution is severe (Amaral-Zettler et al., 2020).

This study aimed to develop a cost-effective methodology to explore critical knowledge gaps regarding the impacts of floating macro(plastic) debris on lower trophic-level organisms in river ecosystems. The experimental design focused on detecting the effects of macro(plastic) debris on plankton communities under specific environmental conditions. By identifying threshold levels of surface macro(plastic) debris that disrupt ecosystems, this framework seeks to inform the development of standardised assessment protocols. A key priority was to create a replicable and adaptable design suitable for remote settings with limited infrastructure.

2.3 Methodology

The original aim of this study was to investigate the species composition and productivity of lower trophic level species in Manado, Indonesia, between ecologically similar sites with contrasting levels of floating plastic pollution. This study would also have aimed to document litter loads and communities associated with biofouled litter samples. Due to COVID-19 fieldwork was limited to the UK. Due to the timing of the phytoplankton bloom and constraints related to boat and laboratory availability, this experiment was conducted under more limited conditions than initially planned.

2.3.1 Experimental design

The basic experimental design involved adding macroplastic at different levels of surface cover to microcosm cells and recording the impact on in-situ phytoplankton communities. The response variables in experiments were phytoplankton concentration and functional group, which can be determined using a basic microscope and with relatively little formal training. Chlorophyll-a concentrations were also explored as a response variable, requiring access to benchtop fluorometry. Experimental microcosms were trialled using plankton samples collected from the Solent estuary, Southampton.

Although studies have demonstrated a high microplastic load in Solent waters (Gallagher et al., 2016), particularly in sea surface microlayer (SML) (Anderson et al., 2018), floating macroplastic load has not been studied in the Solent estuary. Therefore, observed levels of macroplastic debris are low and do not present an obvious environmental concern. The microcosm design proposed in this study is intended to be used in environments with high volumes of surface macro(plastic) debris, to identify a threshold of floating debris where a change in plankton community is easily measurable.

Three experimental variables were considered: plastic type, plastic surface stress loading, and presence or absence of biofouling. Each microcosm was treated with either 50% or 100% surface cover of plastic stress, and one of two plastic types, which were either clean or biofouled treatments that had been exposed to the Solent estuary for a period of 3 months during winter. The main objectives were:

- Provide a methodology to establish a microcosm for naturally occurring plankton assemblages that can be easily replicated and expanded upon to assess impacts of floating macroplastic stress.
- Discuss limitations of methodology and adaptations that could be applied to extend the research.
- Evaluate findings of trial experiment in the light of current knowledge regarding the abundances, distribution and impacts of plastic pollution.

The study aimed to design a reproducible framework for investigating the influence of macroplastic debris loads on ambient planktonic communities. However, several limitations of this trial experiment must be acknowledged:

- The plankton densities sampled to represent the local community in the microcosms were relatively low, originating from a meso-tidal, partially enclosed estuary dominated by diatoms.
- The study examined only a small size fraction of the eukaryotic plankton community.
- The experimental design exclusively focused on plastic materials as treatment substrates, neglecting the comparison with non-plastic materials.
- Higher levels of surface plastic load than normally found in ambient conditions were initially explored to understand if a measurable response could be detected using the simplified approach applied. Surface cover stress should reflect those found in ambient environments in future research.
- Biofouling on exposed plastic samples was inconsistent, and its extent was not quantified prior to their random addition to the microcosms.
- Both clean and biofouled plastics were examined, but this approach could be applied to successional stages of exposed debris loads to explore relevant temporal impacts in more detail.
- While light conditions reflected ambient diurnal patterns, light intensity varied between samples depending on distance from the source.

2.3.2 Treatments

Two plastic materials of high relevance were chosen as stress factors for the trial microcosm experiment. The rationale behind the selection of test substrates was based on considering two of the most widely produced polymers with contrasting qualities: polyethylene terephthalate (PET) and polyvinyl chloride (PVC). PET belongs to the same family of thermoplastics as polyethylene, referred to as polyolefins. These are the most widely produced plastic polymers globally, followed by PVC (Braun, 2001).

PET is often transparent and used in many applications, including food and drinks packaging, which are some of the most abundantly found marine debris items (Addamo et al., 2017; Biermann et al., 2020; Castro-Jiménez et al., 2019). PVC is usually non-transparent and has a wide variety of applications, including construction materials, piping, bags, textiles and automotive materials. Furthermore, vinyl chloride can present a higher toxicity load (Braun, 2001), containing up to 80% additives by weight (van Oers et al., 2012). There is limited scope of existing ecotoxicology experiments related to plastic additives; however, Zimmerman (2019) highlighted the toxicity variations among different polymer types, noting that PVC was found to induce the highest toxicity and PET the lowest.

To test these materials in a traceable form and considering a comparable surface area for these initial test substrates, 2 x 1 mm thick sheets of PET and PVC were sourced from a plastic fabrication company. Two of these sheets (1 x PET and 1 x PVC) were utilised as clean plastic samples, which were cut to size and washed with filtered seawater prior to adding to the microcosms. The other two sheets (1 x PET and 1 x PVC) were attached to a wooden frame and exposed to Southampton Solent water for 3 months (between December and March 2021) by the National Oceanography Centre's harbour (Figure 2.1a), prior to being cut and added to microcosms (Figure 2.1b).

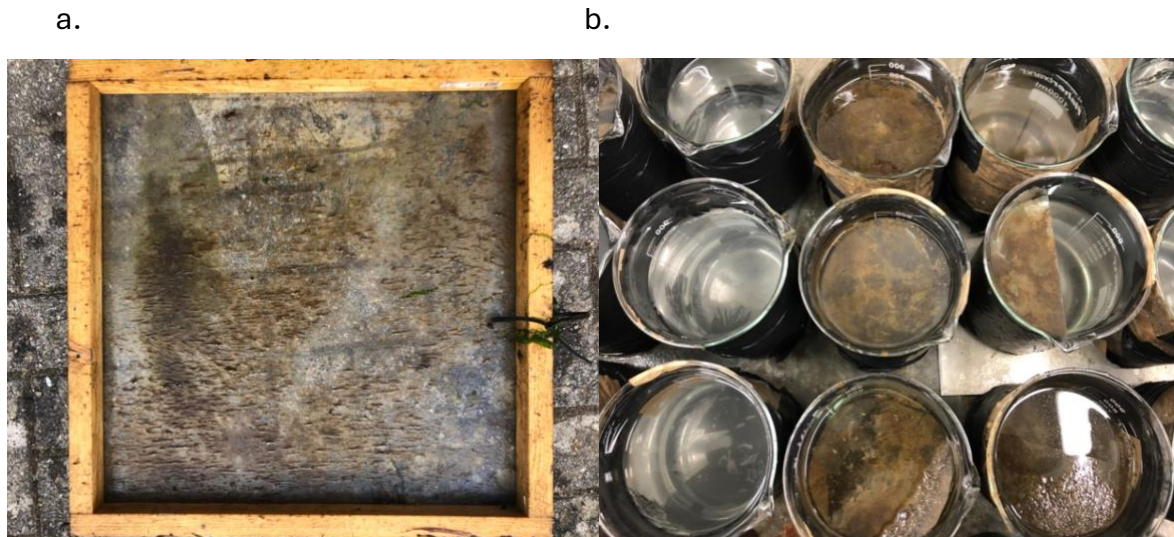


Figure 2.1: Photos of polyethylene terephthalate (PET) sheet exposed to the Solet estuary a) after removal of 3 months exposure and b) after the sheet had been cut and added to microcosms.

Plastic sheets were added to the surface of established microcosms at loads of either 50% or 100% surface cover, resulting in 8 treatment groups (Figure 2.2). Treatment microcosms were assessed in triplicate, alongside an additional 12 control microcosms. Treatment groups were assessed individually and collectively as ‘clean’ and ‘biofouled’ treatments, noting that surface cover varied from 50-100% when grouped.

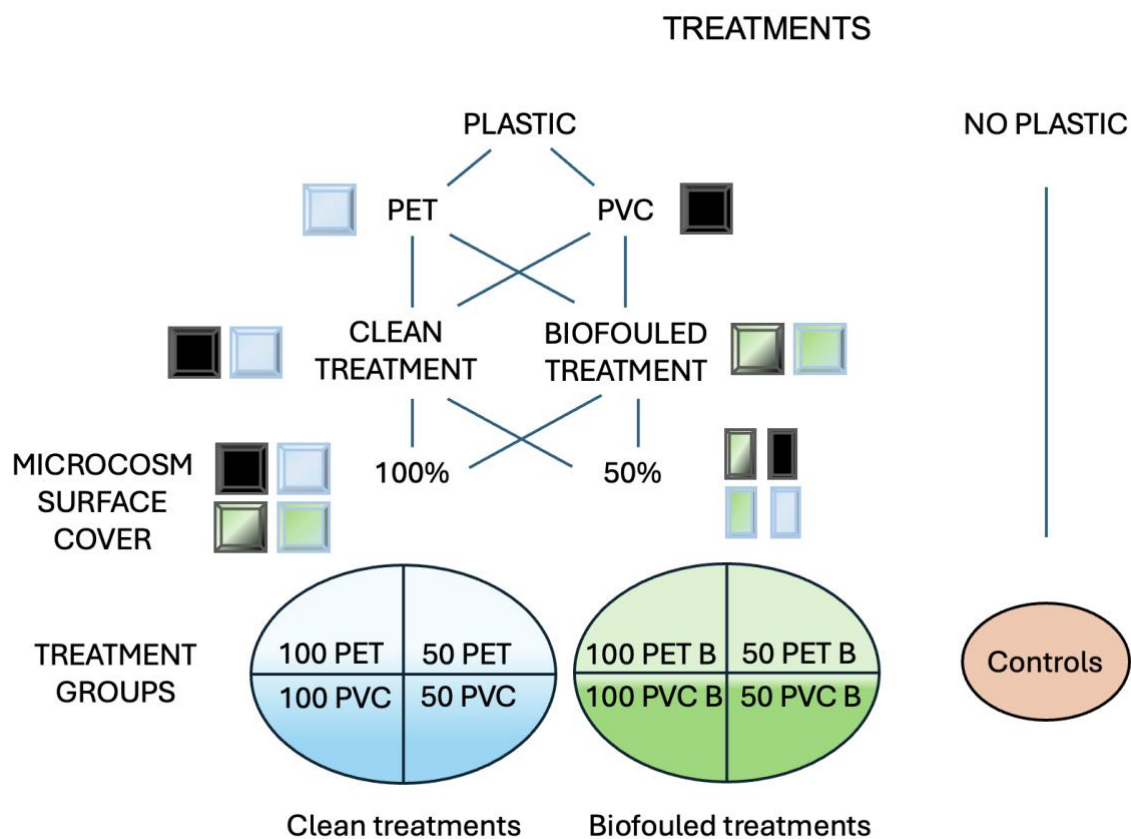


Figure 2.2: Factorial tree diagram of floating debris stress treatment groups and controls utilised in experiment to explore ambient plankton community response in microcosms.

2.3.5 Establishing ambient planktonic community

The source plankton community was collected from the Southampton Water within the Solent, Southampton (Figure 2.3), located on the south coast of the United Kingdom with an estimated catchment area of 1,800 km² (Gallagher, 2016). Southampton water is a large estuary system formed at the confluence of the Rivers Itchen and Test, forming a body of water that envelopes the northern coast of the Isle of Wight, providing two main connections as it mixes with the English Channel.

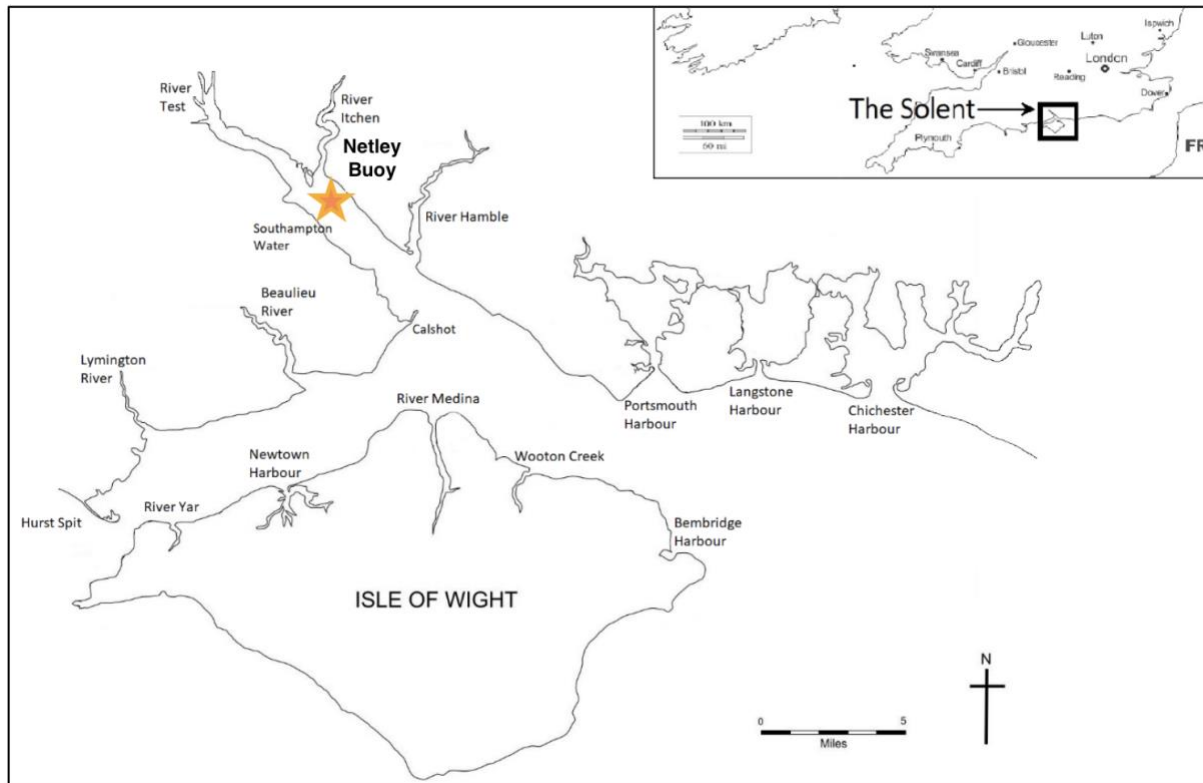


Figure 2.3: Location of Netley Buoy sampling site for plankton within Southampton Water (map from [Parry and Hendy \(2022\)](#)).

The Solent spring bloom of phytoplankton is a major, but short-lived, event in the estuary's calendar. This community is dominated by diatoms and primarily controlled by an increase in irradiance, which subsequently provides a significant boost to the ecosystem's productivity ($>10 \text{ mg/m}^3$ chlorophyll *a*) (Iriarte and Purdie, 2004). The timing of the bloom event varies annually, but has been found to occur approximately 1 week after the mean water column photosynthetic active radiation (PAR) exceeds $380 \text{ h/m}^2/\text{d}$ and prior to this, chlorophyll *a* is limited to $<10 \text{ mg/m}^3$ (Iriarte and Purdie, 2004). Concentrations of nitrate ($\sim 6 \text{ mg/L}$) and phosphate ($\sim 0.3\text{-}0.6 \text{ mg/L}$) (Holley et al., 2007) are relatively low compared to other rivers in the UK (Burt et al., 2011; Edwards and Withers, 2008). While the Solent estuary is not considered eutrophic, it is a high nutrient system that requires monitoring (Xiong, 2000).

A horizontal plankton trawl was conducted on the 2nd of March 2021 at the Netley Buoy ($50:52.3043\text{N}$; $1:22.7339\text{W}$) in the middle of the estuary (Figure 2) between 10:00-11:00 (2nd HW at 12:58, 4.7 m). 10 L of sample was collected using a $100 \mu\text{m}$ plankton net and a plastic collection bottle. 40 L of ambient seawater was also collected and filtered

through a 0.22 µm capsule filter. This water was used to dilute plankton samples across two 30 L opaque plastic buckets with lids.

2.3.6 Establishing microcosm treatments

Samples were transferred back to the laboratory and immediately filtered through a 200 µm mesh to remove the larger size class of the mesozooplankton community and minimise the impact of grazing in the microcosms (Riccardi, 2010). Sample water was then homogenised by stirring slowly for 5 minutes and distributed across 36 x 1 L borosilicate glass beakers, which were wrapped in black plastic to limit light entry. The 1 L glass beakers were filled with 900 mL sample water.

Microcosms were left to settle for 24 hours and then gently mixed with a pipette for 2 minutes before taking each sample. For exploratory purposes, the planktonic response was measured over 10 days to evaluate the possible changes in plankton abundance and diversity (Lafabrie et al., 2013; Othman et al., 2017). Incubation was performed in a temperature-controlled room and measured under controlled conditions, with temperature and light cycles set as closely as possible to the sampling location (12/12 dark light hours at 7 degrees). Non-enriched water samples were used.

The incubation of each plastic treatment group was prepared in triplicate. Due to an inconsistent light source in the trial experiment, controls were prepared and established next to each substrate treatment in triplicate, with 12 control microcosms. In total, 36 plastic samples were hand cut out of the clean and biofilm sheets with a Stanley knife to match the diameter of the glass microcosms using a stencil. These plastic samples were either left whole or cut in half to provide the two treatments for surface level floating plastic stress (Figure 2.1).

A full list of equipment required is provided in Table 2.2. Specific quantities may vary based on the experimental setup and replicates desired. Upon termination of the experiment, protocols for disposal were followed to ensure the safety of both the researchers and the environment. Biological materials and plastic waste were separated into the appropriate waste containers, which are disposed through a licensed waste disposal service following local regulations and guidelines.

Table 2.2: Equipment list for microcosm experiment on floating macroplastic debris.

Equipment	Purpose
1 L Borosilicate glass beakers	Microcosm containers for experimental treatments
Black plastic wrap	Light restriction for microcosm containers
Microscope	Observation and identification of phytoplankton
Plankton net (100 µm)	Collection of plankton from the Solent Estuary
Plastic collection bottle	Container for collecting plankton samples
0.22 µm capsule filter	Filtration of ambient seawater for dilution
200 µm mesh	Filtration to remove larger mesozooplankton
Plastic treatments (from plastic fabrication company)	Test substrates for plastic types
Wooden frame	Support structure for plastic exposure to ambient environment
Stanley knife	Cutting plastic samples to match microcosm diameter
Plastic buckets with lids	Dilution and homogenisation of plankton samples
Fluorometer	Measurement of chlorophyll a concentration
Syringe	Sampling microcosm water for chlorophyll a measurement
Pipettes	Pipettes for measuring sample volumes
Glass Fibre Filters (GFF)	Collection of microcosm samples for chlorophyll a measurement
90% acetone	Solvent for chlorophyll a extraction
Lugol's solution	Fixative for plankton samples
Dark glass storage bottles	To store fixed plankton samples
Light microscope	Observation and identification of plankton
Sedgewick rafter counting chamber	Counting and assessing community structure
Temperature-controlled room/incubator	Maintaining constant temperature for incubation
Statistical software	Analysing taxonomic categories and community structure

2.3.7 Data analysis and statistics

To gauge the microcosm community response to plastic exposure, two key metrics were measured: chlorophyll *a* (Chl.*a*) fluorescence, serving as a proxy for productivity, and cell count, enabling an assessment of growth response and functional diversity. The concentration of Chl.*a* pigment was monitored on days 1, 2, 3, and 9 of the experiment. Cell abundance and taxonomic composition were assessed on days 1 and 7.

Data analysis and management were conducted using Microsoft Excel and R. Normality of data was assessed through a Shapiro-Wilk test and homogeneity of variances were evaluated using Levene's test. Subsequently, Analysis of Variance (ANOVA), repeated measures ANOVA, a *t*-test, a Wilcoxon-signed rank test or linear modelling was performed to analyse responses across controls and treatment groups. A forest plot was used to visualise significant values of Chl.*a* across treatment groups in comparison to the control group (Chang et al., 2022). In this diagram, if the confidence interval crosses the central (null) line, the effect is not statistically significant. If the reported value is to the right of the null line, it indicates a positive or an increased effect, while if the reported value is to the left of the null line, it indicates a negative or decreased effect.

Among-treatment variability in plankton community composition was visualised using principal component analysis (PCA) to help interpret similarities and dissimilarities in the microcosm communities. Analyses were performed using the Bray-Curtis approach and the taxonomic category for cell counts, producing groups of microcosms based on clean and biofilm plastic treatments.

2.3.7.1 Productivity

Microcosm samples were taken near the expected chlorophyll maximum at 10:00 am on day 1, day 2, day 3, and day 9 of the experiment. Chl.*a* was measured using a Turner Design model 10-AU benchtop fluorometry device, which has a detection range of 0.025-2.0 µg/L with a relative error of <3 % (Welschmeyer, 1994). Less than 25% of the

total volume was removed throughout the experiment for analysis and no nutrients were added to incubations to allow for growth under natural levels.

Microcosms were gently mixed with a pipette for 2 minutes before sampling from the centre of the container to ensure even distribution of cells. A 10 mL sample of each treatment and control were taken via a syringe onto a glass fibre filter (GFF). Chl.a was extracted by adding the GFF to 7 mL of 90% acetone and stored in the freezer overnight (Su et al., 2010). To calculate the concentration of Chl.a ($\mu\text{g/L}$) based on the reading from the fluorometer, the following formula was used:

$$\text{Chlorophyll } a \text{ concentration } (\mu\text{g/L}) = \left(\frac{\text{Fluorometer reading } (\mu\text{g/L})}{\text{Volume of acetone used (L)}} \right) \times \left(\frac{\text{Total volume filtered (L)}}{\text{Volume filtered onto GFF filter (L)}} \right)$$

Substituting the volumes described above provides the formula:

$$\text{Chlorophyll } a \text{ concentration } (\mu\text{g/L}) = \left(\frac{\text{Fluorometer reading } (\mu\text{g/L})}{0.007 \text{ L}} \right) \times \left(\frac{0.01 \text{ L}}{0.01 \text{ L}} \right)$$

Treatment and day were subsequently applied as fixed effects in a linear mixed-effects model. The model formula included sample number as a random effect:

$$\text{Concentration } (\mu\text{g/L}) \sim \text{Day} * \text{Treatment} + (1 \mid \text{Sample Number})$$

2.3.7.2 Community structure

Samples were taken on day 1 and 7 of the experiment to investigate plankton abundance and community assemblage using microscopy. For each microcosm, 20 mL of sample was obtained using a syringe and fixed with 0.5 mL Lugol's solution in a dark storage bottle. Samples were left in the dark for 24 hours. Plankton counts and identification were performed using a binocular light microscope and Sedgewick Rafter Counting Chamber (50 x 20 x 1 mm). At least 400 cells were counted for each sample to ensure 10-20% standard error. To estimate the concentration of cells, the following formula was used:

$$\text{Cell concentration (cells/L)} = \left(\frac{\text{Number of cells counted}}{\text{Counting area (mm}^2\text{)}} \right) \times \left(\frac{1}{\text{Depth of counting chamber (mm)}} \right) \\ \times \left(\frac{1}{\text{Dilution factor}} \right) \times 1000$$

Species richness (S) was calculated as the number of taxa identified in each sample. The Shannon-Weaver diversity index (H') was applied to assess the diversity in microcosm communities. The following formula was used to calculate the diversity index, where P_i is the proportion of individuals belonging to the i -th species:

$$H = - \sum_{i=1}^S p_i \ln(p_i)$$

P_i was calculated by dividing the count of individuals for each species by the total number of individuals (N). The Shannon Equitability Index (E_H) was applied to assess evenness using the following equation:

$$E_H = \frac{H}{\ln(S)}$$

2.4 Results

2.4.1 Biofilm development

After 3 months, a biofilm formation with patchy coverage was observable over both the PET and PVC plastic sheets. Figure 2.4 provides a summary timeline of the biofouling process observed on plastic sheets exposed in Southampton Water. Microorganisms were quick to establish an initial matrix of extracellular polymeric substances (EPS), which subsequently enabled the attachment of larger organisms, such as molluscs and filamentous brown algae.

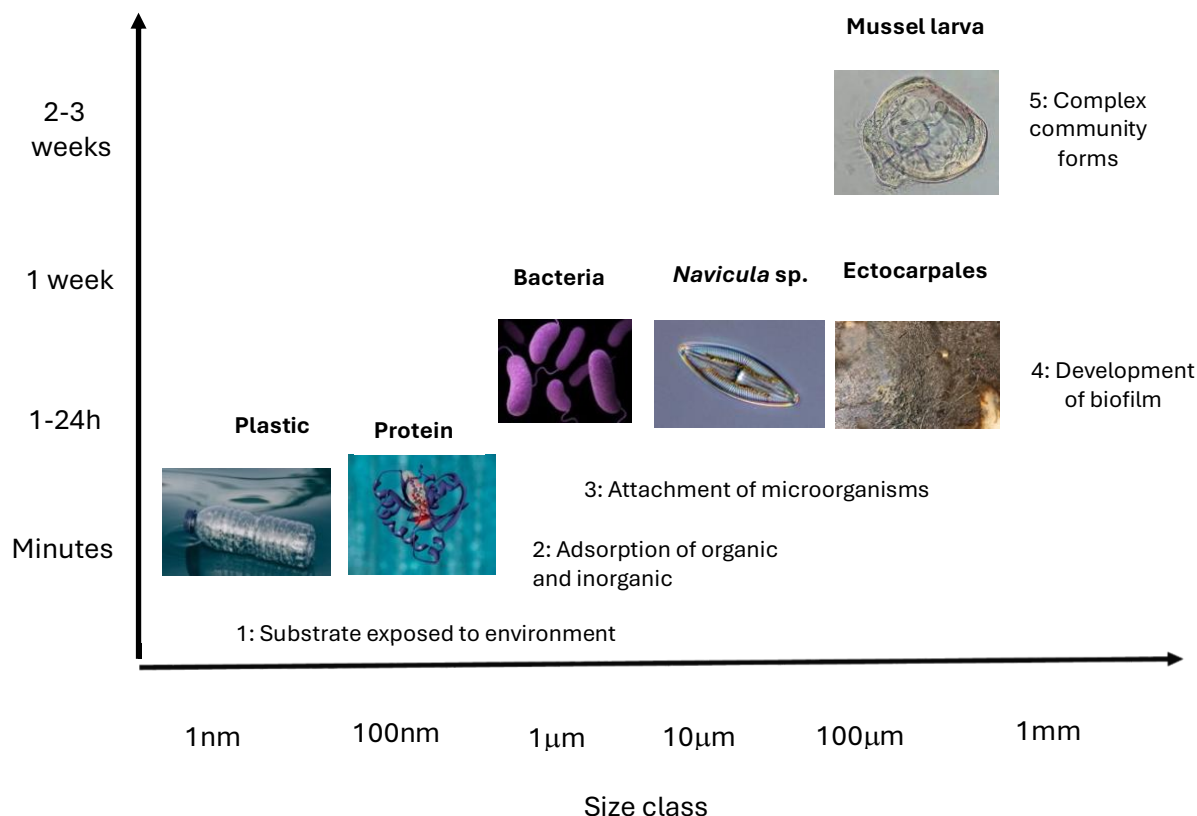


Figure 2.4: Timeline of biofilm development observed on plastic sheets exposed to the Solent estuary for 3 months.

2.4.2 Chlorophyll a trend across microcosms

The estuarine water had a salinity of 27‰. Chl.a concentrations in microcosms on day 1 (prior to treatment) ranged from 0.02-0.08 μg/L (Figure 2.5a), with an average of 0.04 μg/L ± 0.00 (SE). A comprehensive table detailing Chl.a values of microcosms prior to treatment is provided in Appendix I (Table 1). A Shapiro-Wilks test confirmed a normal distribution across all microcosm treatment groups on day 1 (Appendix I, Table 1) and a Levene's test for homogeneity of variances based on the mean yielded a non-significant result ($F_{8,27} = 1.70$, $p = 0.14$), indicating equal variances across groups. ANOVA reported no significant differences between groups ($F_{8,27} = 1.76$, $p = 0.13$).

Chl.a values in control microcosms were not normally distributed by day 9 of the experiment ($W = 0.59$, $p < 0.01$). A Wilcoxon signed-rank test revealed a significant difference between day 1 and day 9 ($V = 0$, $p < 0.01$), with a mean Chl.a concentration of 0.22 μg/L ± 0.07 (SE) on day 9.

In contrast, Chl.a values in microcosms exposed to clean plastic samples followed a normal distribution on day 9 ($W = 0.98$, $p = 0.99$), while a Levene's test reported unequal variance ($F = 40.44$, $p = <0.01$). A Wilcoxon signed-rank test also revealed a significant difference between day 1 and day 9 ($V = 0$, $p = <0.01$).

For microcosms exposed to biofouled plastic samples, Chl.a values were not normally distributed by day 9 ($W = 0.89$, $p = 0.13$). A Wilcoxon signed-rank test also detected a significant difference between day 1 and day 9 ($V = 0$, $p = <0.01$), with a mean Chl.a value of $2.82 \mu\text{g/L} \pm 0.78$ (SE) on day 9 (Figure 2.5b).

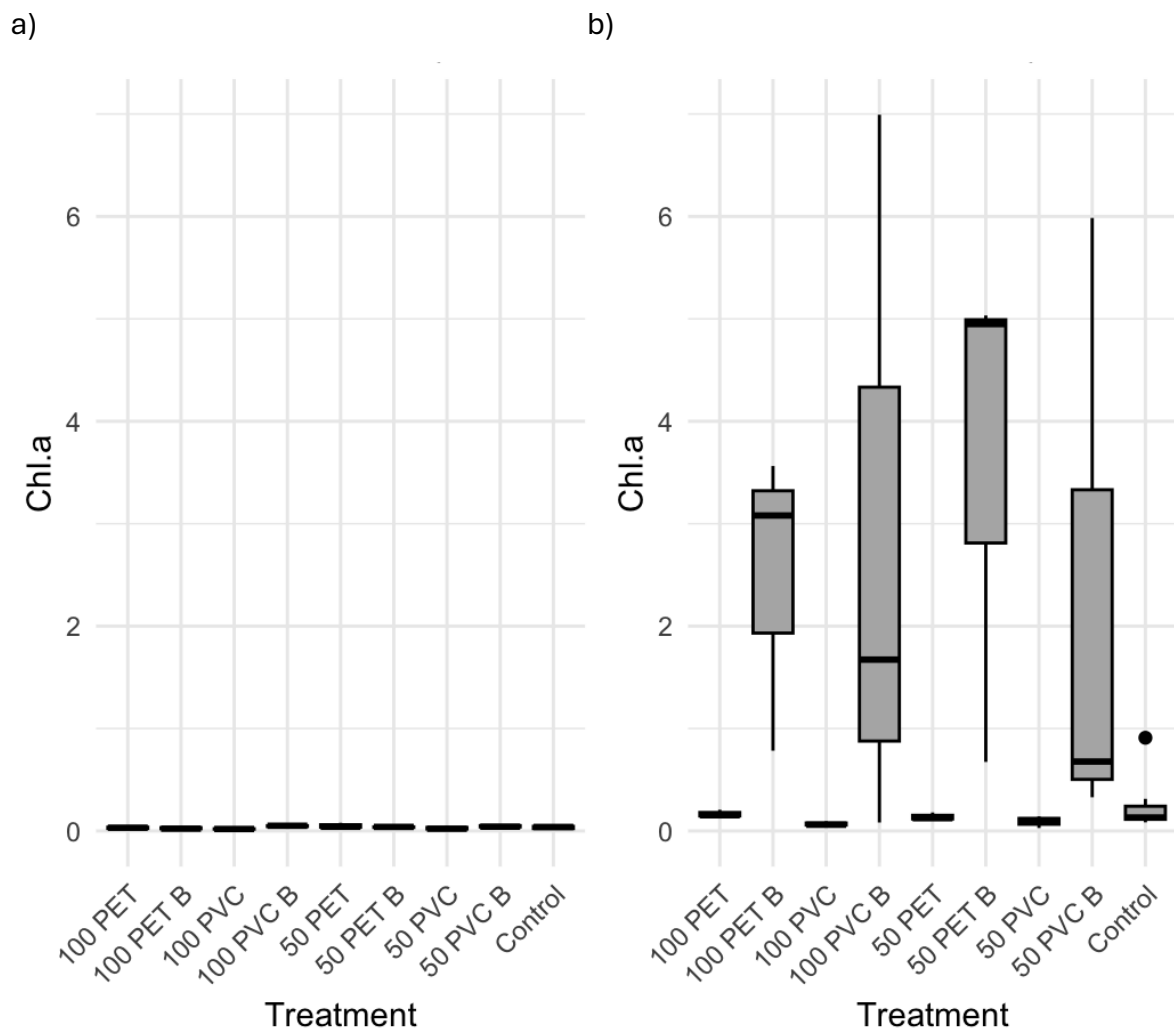


Figure 2.5: Box plots of chlorophyll a (Chl.a) concentration (ug/L) measurements on a) day 1 and b) day 9 of the experiment across microcosms in triplicate treatment groups of clean polyethylene terephthalate (PET) and polyvinyl chloride (PVC) samples at 100% and 50%

surface coverage stress, biofouled (B) PET and PVC samples at 100% and 50% surface coverage stress, and controls ($N = 12$).

Twelve control microcosms demonstrated a sustained increase in Chl.a throughout the experiment, reporting an average Chl.a value of $0.04 \mu\text{g/L} \pm 0.00$ (SE) on day 1 and $0.22 \mu\text{g/L} \pm 0.04$ (SE) on day 9 (Figure 2.6). Linear regression reports a statistically significant relationship between day and Chl.a ($F_{1,46} = 31.52$, $p = <0.01$), although a weak correlation ($r^2 = 0.4$) is observed overall. At an individual sample level, these correlations are stronger, with r^2 values ranging from 0.57-0.98 (Appendix I, Table 2).

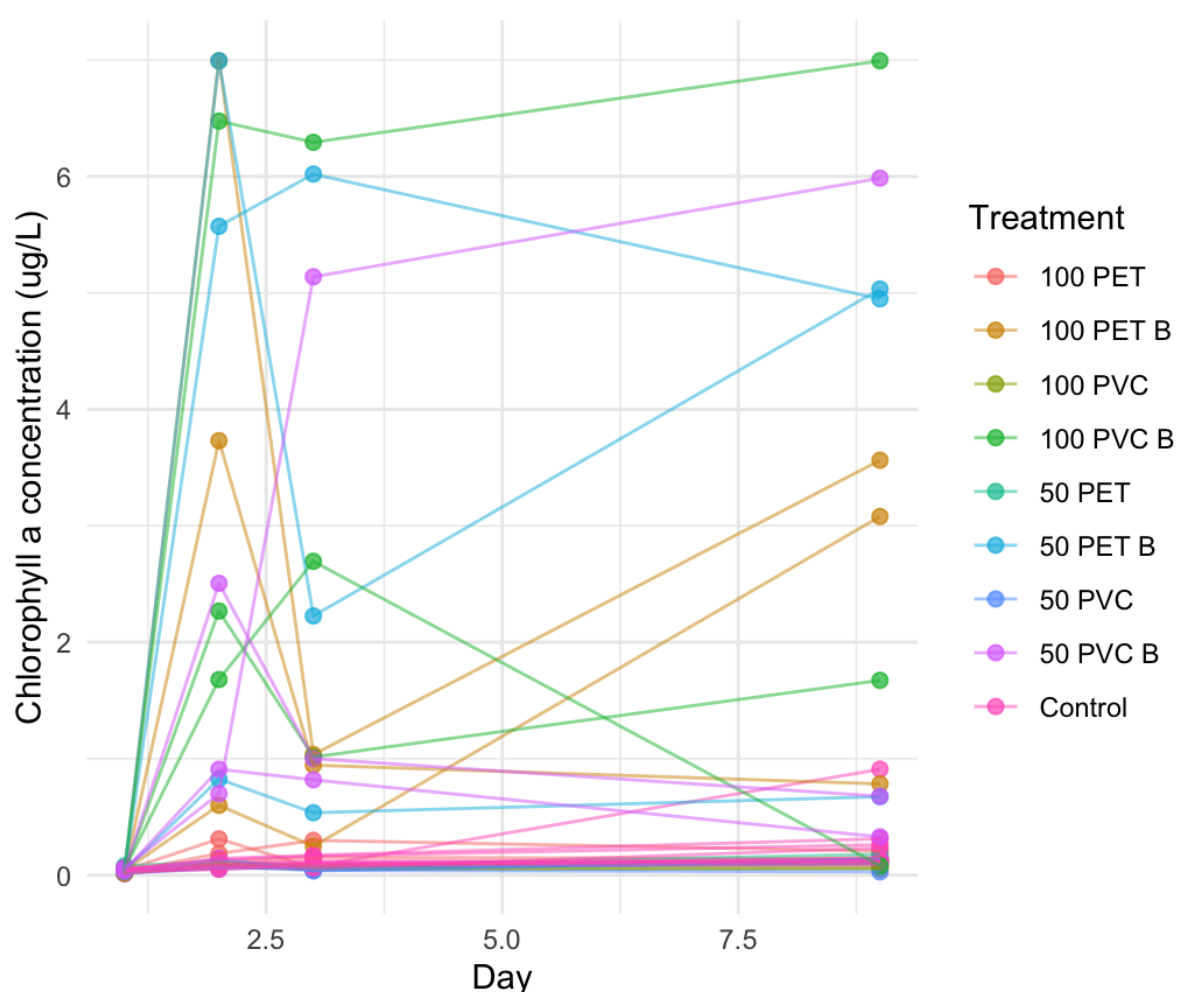


Figure 2.6: Chlorophyll a ($\mu\text{g/L}$) measurements across microcosms in triplicate treatment groups of clean polyethylene terephthalate (PET) and polyvinyl chloride (PVC) samples at 100% and 50% surface coverage stress, biofouled (B) PET and PVC samples at 100% and 50% surface coverage stress, and controls ($N = 12$) for duration of experiment.

Clean plastic samples demonstrated an initial increase in Chl.a, with a mean of $0.03 \mu\text{g/L} \pm 0.00 \text{ (SE)}$ day 1 to $0.12 \mu\text{g/L} \pm 0.06 \text{ (SE)}$ on day 2. This was followed by a decreasing trend for 100 PET and 100 PVC microcosms for the duration of the experiment. The 50 PET and 50 PVC microcosms also demonstrate a decreasing trend following day 2 but recover slightly by day 9. Biofouled plastic samples demonstrate the largest observed increase in Chl.a concentrations, reporting an average of $3.27 \mu\text{g/L} \pm 0.38 \text{ (SE)}$ on day 2, which decreased to $2.33 \mu\text{g/L} \pm 0.33 \text{ (SE)}$ on day 3.

Regression coefficients determined from linear mixed-effect modelling varied across treatments (Figure 2.7). Both intercepts and slopes are higher in treatments with biofilm present. Both the PET and PVC biofilm treatments produced significantly higher intercept terms at 50% and 100% plastic loading, respectively, indicating a positive effect on Chl.a concentrations. No other significant differences were noted among plastic treatment types or loadings.

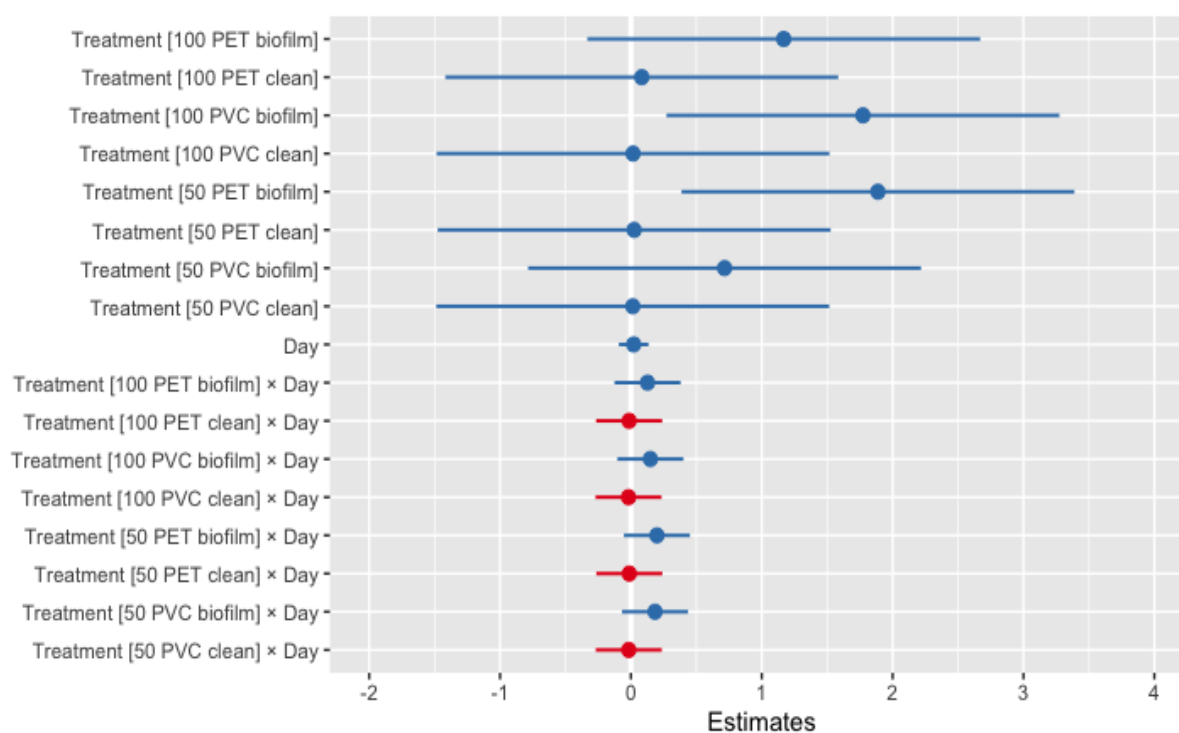
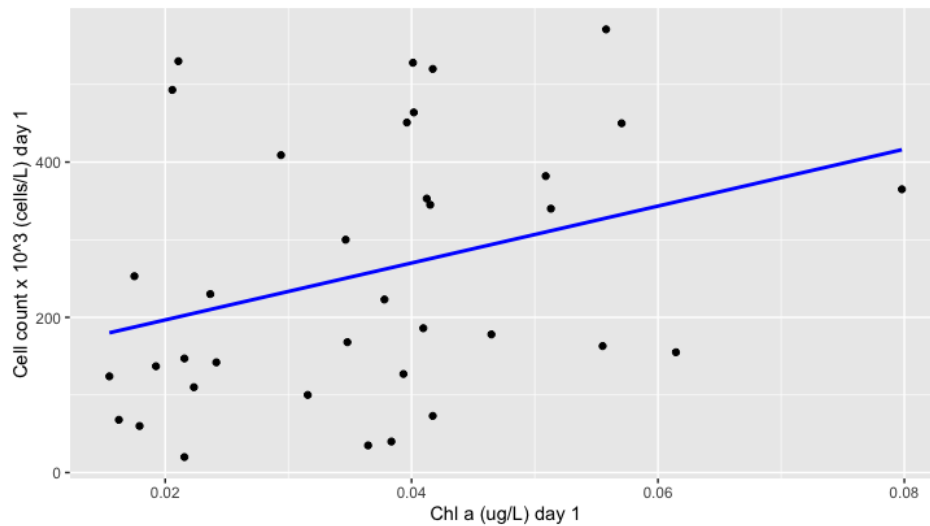


Figure 2.7: Forest plot with regression coefficients for chlorophyll a, with a line through each point representing the confidence interval. In contrast to control treatments, positive coefficients are highlighted in blue and negative coefficients are highlighted in red.

2.4.5 Cell density and chlorophyll a

Cell count and Chl.a measurements in theory both assess the concentration of photosynthetic phytoplankton. At the start of the experiment, relatively low cell concentrations led to poor correspondence between the two measurements, however by day 7, both measures were reported to covary ($r^2 = 0.7$) (Figure 2.8).

a.



b.

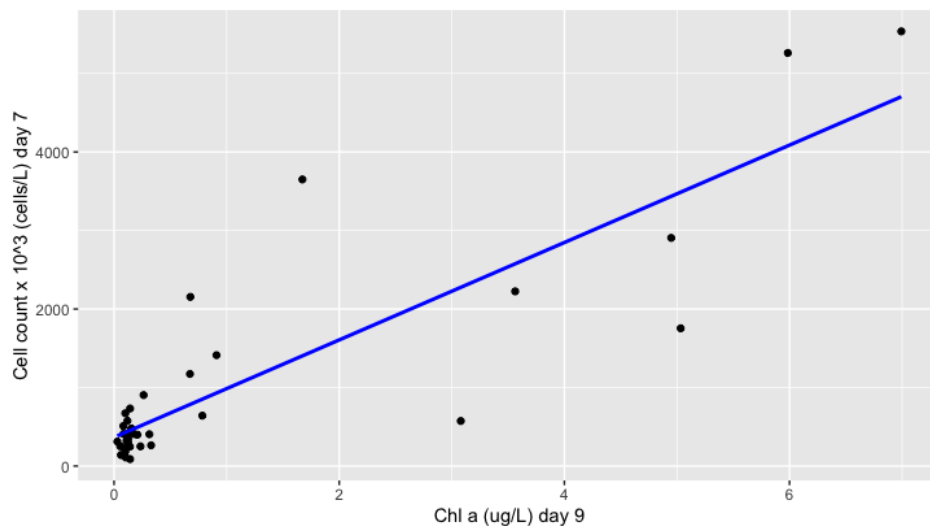
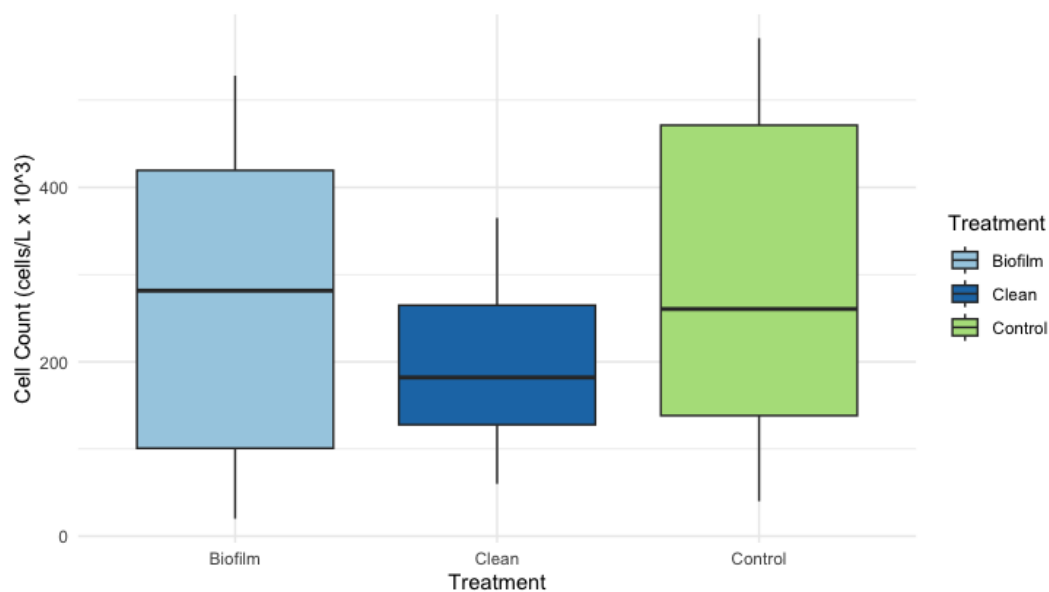


Figure 2.8: Scatterplots of cell count and chlorophyll a (log scale) on a) day 1 and b) day 7 and 9 (note difference in y-axis).

Average phytoplankton cell density in control microcosms increased from 306.5×10^3 cells/L ($SE = 56.27 \times 10^3$) on day 1 to 530.17×10^3 cells/L ($SE = 80.18 \times 10^3$) on day 7

(Figure 2.9). A Shapiro-Wilk test of normality revealed no significant departure from normal distribution across control treatments for day 1 ($W_{12} = 0.87, p = 0.07$) or day 7 ($W_{12} = 0.87, p = 0.06$). Levene's test of variance also found no departure across control treatments for day 1 or day 7 ($F_{1,22} = 1.39, p = 0.25$). A subsequent one-tailed paired sample t -test reported a significant difference between day 1 and day 7 across control microcosms ($T_{10} = -2.61, p = 0.01$).

a.



b.

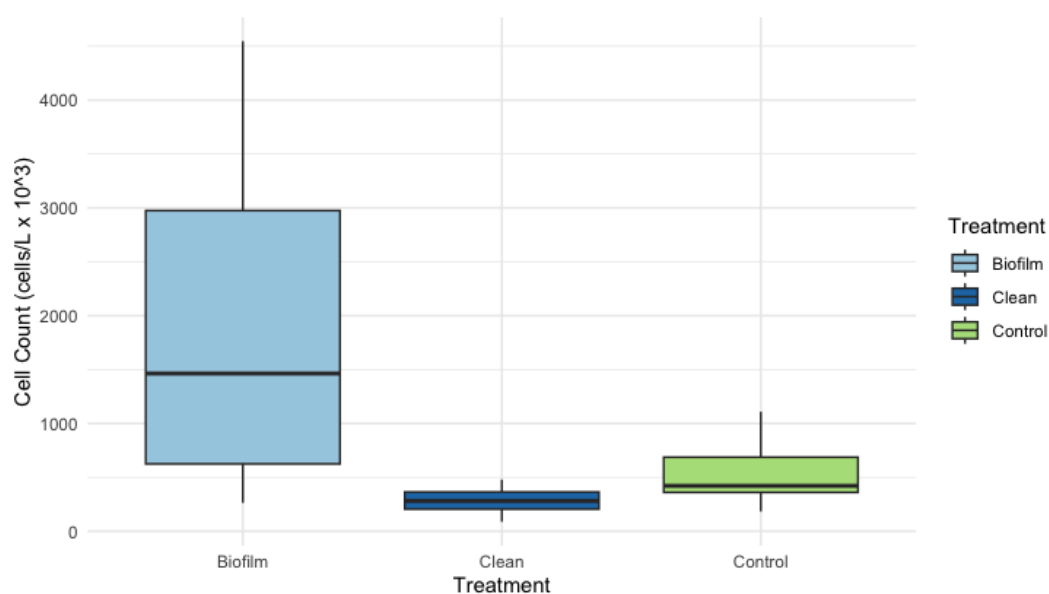


Figure 2.9: Box plots of cell count (cells/L $\times 10^3$) across treatments (grouped into clean, biofilm and control groups) on a) day 1 and b) day 7 of the experiment (note difference in y-axis)

Considering clean plastic treatments on day 1, phytoplankton cell densities ranged from an average of 131.67×10^3 cells/L ($SE = 35.32 \times 10^3$) in 50 PVC microcosms to 291×10^3 cells/L ($SE = 52.31 \times 10^3$) in 50 PET microcosms prior to treatment. On day 7, cell densities ranged from 173×10^3 cells/L ($SE = 71.09 \times 10^3$) for 50 PVC to 375×10^3 cells/L ($SE = 68.04 \times 10^3$) for 100 PET. A Shapiro-Wilk test for normality found no departure across clean plastic treatments for day 1 or day 7 (Appendix I, Table 3). A Levene's test reported no difference in variance as a function of treatment for day 1 ($F_{3,8} = 0.27$, $p = 0.84$) or day 7 ($F_{3,8} = 0.37$, $p = 0.78$). A repeated measures ANOVA reported a significant difference between day 1 and 7 across treatments ($F_{3,1} = 13.79$, $p = 0.03$).

Concerning microcosms treated with exposed plastic, cell counts ranged from an average of 67.67×10^3 cells/L ($SE = 26.12 \times 10^3$) for 100 PET to 471×10^3 cells/L ($SE = 34.84 \times 10^3$) for 50 PET on day 1 (Appendix I, Table 4). On day 7, counts ranged from $1,117.33 \times 10^3$ cells/L ($SE = 472.74 \times 10^3$) for 100 PET to $3,012 \times 10^3$ cells/L ($SE = 1,245.64 \times 10^3$) for 100 PVC on day 7. A Shapiro-Wilk test for normality found no departure from normality across all biofilm plastic treatments for day 1 or day 7 (Appendix I, Table 3). A Levene's test reported equal variance on day 1 ($F_{3,8} = 0.53$, $p = 0.68$) and day 7 ($F_{3,8} = 0.37$, $p = 0.78$). A repeated measures ANOVA reported a significant difference between day 1 and 7 across treatments ($F_{3,1} = 17.91$, $p = 0.24$).

2.4.6 Plankton community composition

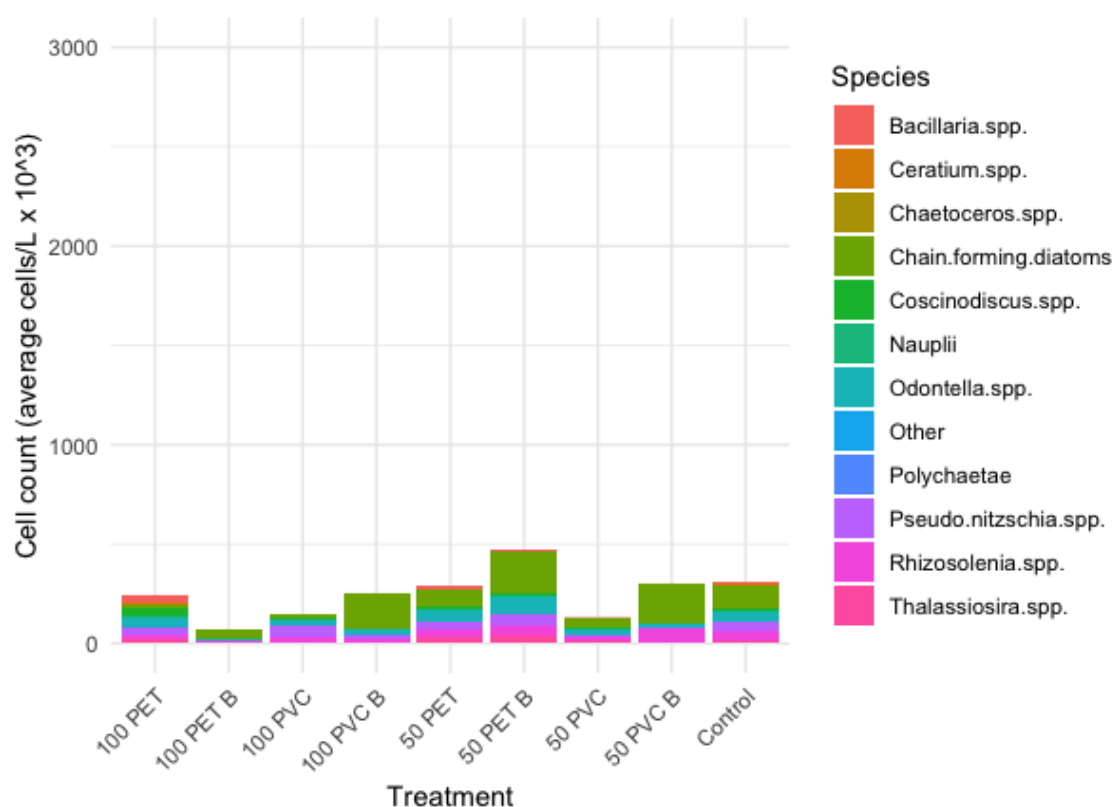
Over the duration of the experiment the most dominant taxa identified were diatoms, belonging to the Class Bacillariophyceae, comprising 98% of organisms counted on day 1 and day 7 of the experiment (Figure 2.10). Initial cell density across all microcosms was dominated by centric diatoms (67 %), particularly chain forming diatoms of the Subclass Coscinodiscus, which contributed 41 % of cells identified across all microcosms prior to treatment on day 1.

In the control microcosms, centric diatoms dominated the assemblage on day 1 (65%), followed by pennate diatoms (33%). On day 7, this proportion remained similar with 64% centric and 35% pennate diatoms (Figure 2.10). Species richness remained high at a value of 10 on day 1 and day 7, although diversity and evenness reported slightly lower

values on day 7 (Appendix I, Table 4). Across the clean plastic treatments on day 1, 57% and 39% were centric and pennate, respectively. On day 7, 56% of diatoms were centric, with chain-forming species forming a larger proportion than day 1.

Prior to treatment, biofilm plastic microcosms were dominated by centric diatoms (87%), which were mainly chain forming species (57%), followed by pennate diatoms (22%). On day 7, pennate diatoms dominated 86% of the cell counts. Notably, *Navicula* spp., *Skeletonema* spp. and *Thalassionema* spp. were the additional species observed on day 7 (Figure 2.10). Prokaryotes were also observed in 75% of biofilm microcosm samples on day 7. Species richness appeared to increase in across all biofilm treatment except 100 PVC, but the diversity and evenness indices either stayed the same or lowered across treatments (Appendix I, Table 4).

a.



b.

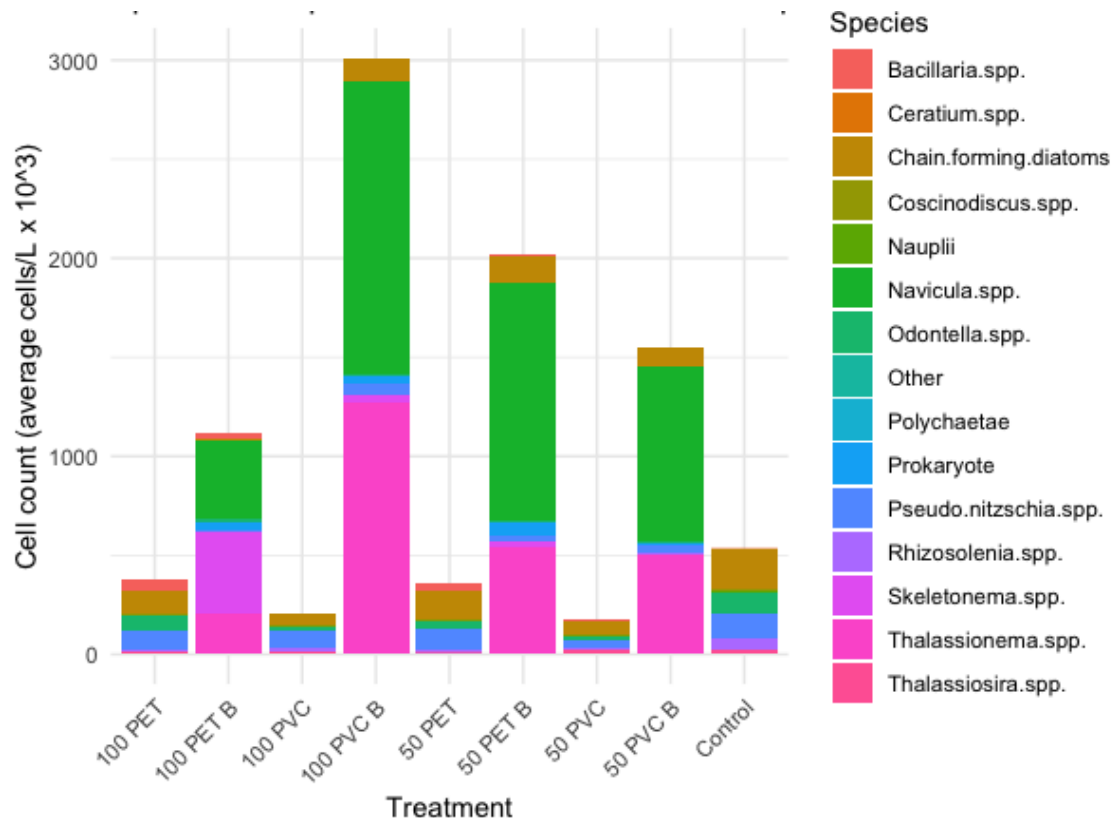


Figure 2.10: Bar charts illustrating planktonic taxa identified (average cells/L $\times 10^3$) across microcosms on a) day 1 and b) day 7 of the experiment.

2.4.7 Similarities and dissimilarities

The Principal Component Analysis (PCA) vector plot for day 7 (Figure 2.11) illustrates how PC2 accounts for most of the total variance in the analysis of taxonomic categories associated with clean plastic treatments. Associations are apparent between PC1 and biofilm treatments, particularly the PVC treatments (Figure 2.11). The correlation coefficients demonstrate the highest values with *Navicula* and *Thalassionema* spp. for PC2, and *Thalassiosira*, *Coscinodiscus*, and *Odontella* species for PC1 (Appendix I, Table 5).

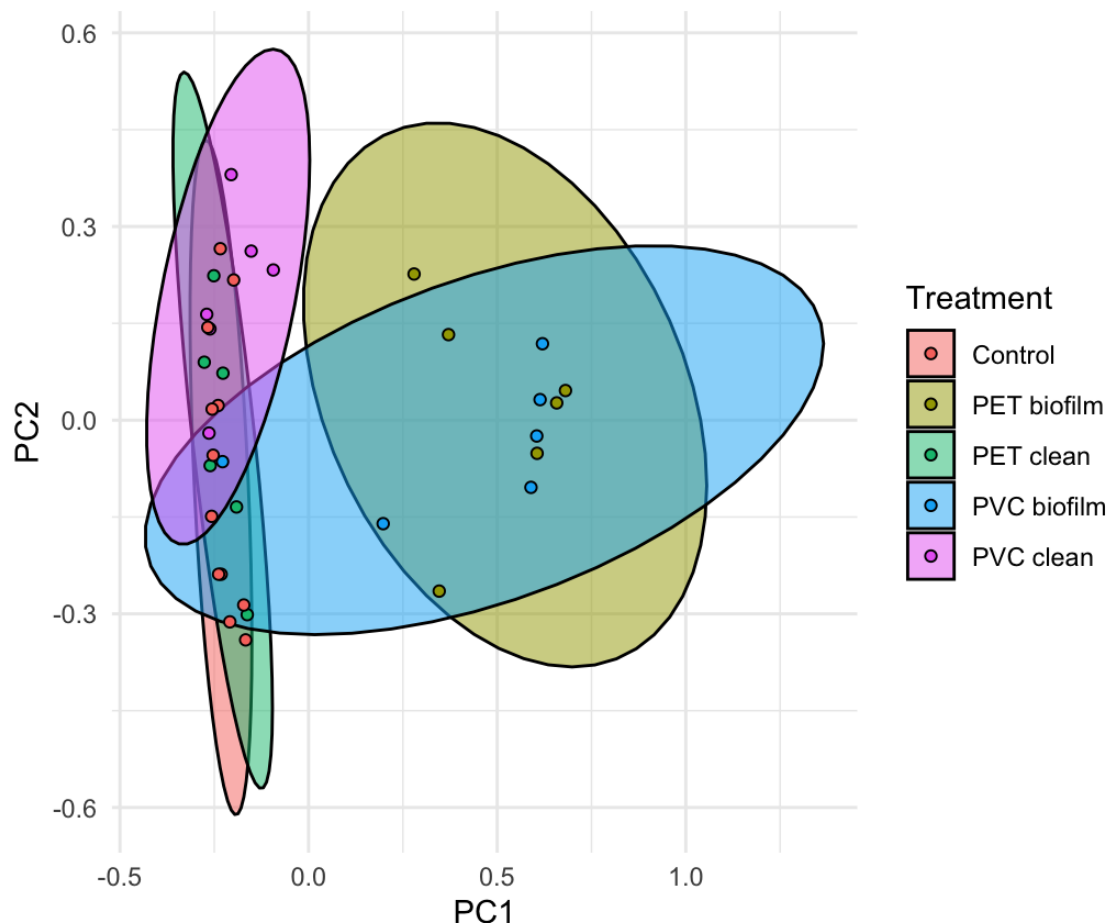


Figure 2.11: PCA of taxa groups identified (cells/mL) combined by polymer type on day 7.

2.5 Discussion

2.5.1 Summary of experimental results

The experimental procedure employed in this study sought to assess the application of a novel and simple experimental microcosm protocol, specifically to explore the impact of different plastic treatments on Chl.a concentrations, cell count and functional diversity within a local context. The results obtained clearly demonstrate the capacity to detect responses in plankton communities over the duration of the experiment.

In the control microcosms, a sustained increase in Chl.a was observed throughout the experiment. The linear mixed effects (lme) model indicated a hindering effect on plankton production from clean plastic treatments, particularly those with 100% and

50% surface cover. This finding suggests that the presence of clean plastic may limit Chl.a production, possibly due to interference with light availability or other environmental factors.

In contrast, plastic with an associated biofilm had a positive influence on Chl.a, However, caution is warranted as the samples demonstrate a wide confidence interval, and significance was only apparent for specific treatments, such as 100% PVC and 50% PET microcosms. This is partly attributable to the patchy colonisation of plastic debris, which is challenging to quantify using a simple approach (Gil and Pfaller, 2016), but should be considered in more detail in future studies. The extremely high values of Chl.a ($>2.0 \mu\text{g/L}$) that exceeded the fluorometer range are likely influenced by larger macrophyte cells that have shed from the biofilm, which challenges the robustness of the observed positive trend in Chl.a for phytoplankton cells in biofilm treatments.

The low initial phytoplankton densities, coupled with testing the lower end of the fluorometer range ($0.025 \mu\text{g/L}$) and natural discrepancies between cell size and Chl.a (Fujiki and Taguchi, 2002; Harrison et al., 2015) may explain the lack of correlation between cell counts and Chl.a values observed on day 1 compared to subsequent days. Diatom cell volumes pose the largest source of variation in community carbon estimates, with even species-specific biovolumes varying up to an order of magnitude (Harrison et al., 2015). However, small cells have a higher carbon density than large cells, therefore biovolume varies several fold while carbon density varies a small amount (Harrison et al., 2015). Human errors in microscopy and poor mixing during the sampling process are also likely to add error to the dataset (Bradie et al., 2018). The weak correlation between cell counts and Chl.a measurements on different days (day 7 and day 9) further underscore the challenges in reconciling results obtained through different analytical techniques.

A significant increase in cell count between day 1 and day 7 was observed for clean plastic treatments ($F_{3,1} = 13.79$, $p = 0.03$). The shift in species composition from large centric diatoms dominating on day 1, to smaller chain-forming species on day 7 may explain this result, which conflicts with Chl.a data. This emphasises the importance of considering both Chl.a and community composition when interpreting results.

The introduction of biofilm treatments markedly altered both Chl.a concentrations and cell count, with small pennate diatoms and chain-forming species dominating the microcosms post-treatment. The identification of diatom groups that were not documented in ambient samples included benthic diatoms (*Navicula* spp.), those associated with hypoxic events (*Skeletonema* spp.), and those associated with high productivity (*Thalassionema* spp.). This highlights the potential influence of biofilm plastic treatments on phytoplankton community composition, at least in the local context under study (Harrison et al., 2015; Hasle et al., 1996; Kobayashi and Takahashi, 2002). Additionally, the identification of large prokaryotes in 75% of samples underscores the pathogenic risk associated with plastic biofilms (Barnes et al., 2009; Jeong et al., 2023; Kirstein et al., 2019; Zettler et al., 2013).

2.5.2 Evaluation of experimental design

The experimental design presented in this study aimed to investigate the potential ecological impacts of plastic pollution on lower trophic level species. The methodological approach developed sought to create microcosms that simulate natural plankton assemblages, offering a novel and practical framework for investigating the influence of surface macroplastic loads throughout different stages of environmental exposure. The design's objectives were to establish replicable, scalable, and robust experimental setups, providing a foundation to explore threshold levels of surface macroplastic that may affect ambient ecosystems, initially exploring both clean and biofouled plastic.

The approach successfully achieved its goals by generating microcosms with comparable ambient plankton samples and yielding measurable results. The study identified notable effects of plastic treatments on phytoplankton productivity. Clean plastic treatments demonstrated measurable alterations, while biofouled plastic treatments induced substantial changes, introducing significant numbers of opportunistic species in all but one of the treated microcosms. These findings provide valuable insights into how high surface macroplastic loads could impact productivity and community structure in low-productivity environments, particularly when plastic has been subjected to biofilm growth.

The observed variability in productivity and cell abundance across biofilm treatments is likely attributed to the patchy colonisation of floating plastic, a phenomenon challenging to quantify cost-effectively. The use of plastic sheets ensured equal surface areas among samples, addressing a methodological challenge, yet this will be an inherent difficulty when exploring materials with differing surface areas.

While acknowledging these achievements, the study recognised limitations that warrant consideration in future research. The trial experiment focused on high surface loads (50% and 100% cover), reflective of highly impacted environments or future scenarios, emphasising the need for future studies to tailor surface plastic loads to local environments, giving priority to vulnerable ecosystems. Additionally, the oversight of sampling the spring bloom in the Solent estuary highlights the importance of incorporating higher ambient plankton densities in future research.

The study's taxonomic resolution was low, but even at this level, introduced genus groups were identified, altering community structure. Some of these groups shared characteristics associated with eutrophic systems, raising questions about the poorly explored link between plastic and eutrophication processes (Zhang et al., 2020). The study underscored the importance of coupling microscopy with fluorescence measurements for community-level impact assessment, advocating for its adoption by academic and research groups with basic training.

The experiment faced challenges in equipment availability, limiting plankton sampling to the 100-200 μm range. Future research may need to consider the microplankton range (10-200 μm), understanding the associated challenges in microscopic identification. To establish a standardised approach, agreement on quantification and categorisation standards are imperative. Equipment limitations extended to the availability of a measurable light source.

2.5.3 Future experimental considerations

The study's overarching objectives aspired to lay the foundations for a robust experimental framework capable of generating standardised insights into the ecological impacts of floating macroplastic on natural plankton communities and ecosystem

functioning. In proposing a basic standardised framework, the study envisioned providing academic and research groups with limited resources a baseline for comparable information, fostering a stakeholder network for collaborative extension and refinement of research.

Moving forward, several considerations and potential improvements emerge to enhance the robustness and applicability of this approach, including the consideration of temperature-controlled rooms or water baths with measurable light sources. While measuring light penetration in microcosms would be ideal, cost efficiency should be weighed against necessity.

Factors influencing the development and composition of biofilm communities are still poorly understood (Pinto et al., 2019; Wright et al., 2020). This study acknowledges the importance of biofilm communities on plastic debris, but future experiments should aim for a more detailed characterisation of the communities and productivity on biofouled samples themselves. Measuring the extent of biofilm growth on samples using a staining assay and subsequent decrease in light transmittance through samples over time, as described by Nelson et al., (2021), could be an additional parameter that would complement this dataset, cost permitting.

There are limited studies comparing biofilm development on different plastic materials across different seasons in the same environment (Misic and Covazzi Harriague, 2019; Oberbeckmann et al., 2016, 2014). The range of plastic materials studied for biofilm development are also limited, with few studies considering abundant floating debris, such as fishing gear and rope items (De Tender et al., 2017; Enrichetti et al., 2021). The inclusion of relevant plastic materials, with environmentally relevant concentrations and appropriate controls is paramount in future experimental design. Additional non-plastic substrate controls would be able to help assess the potential for substrate-specific effects. To capture the temporal variability in estuarine ecosystems, future studies should also explore different successional stages at various times of the year. Seasonal dynamics may influence the response of plankton communities to plastic pollution, and understanding these variations is crucial for developing a comprehensive understanding of the long-term impacts. This will strengthen the reliability of observed

effects and ensure that any changes observed can be confidently attributed to the presence of plastic debris.

To enhance the ecological relevance of this research, it is crucial to align the experimental approach with ongoing environmental monitoring efforts. Studying floating macroplastics in rivers and other hotspot areas would provide valuable context, allowing for a better understanding of the relevant floating loads, residence times, and potential sources of plastic pollution. Incorporating seasonal studies on biofouling dynamics and sinking rates of different plastic types would also contribute valuable information to our understanding of the mechanistic pathways of plastic within aquatic environments.

While this study focused on community abundance and productivity, future work should prioritise extending knowledge regarding the ecosystem-level impacts of plastic pollution. This includes assessing the effects on the carbon budget and biodiversity of plankton communities, providing a more comprehensive understanding of the cascading effects of plastic pollution within aquatic ecosystems. To facilitate collaboration and data synthesis across studies, it is imperative to develop harmonised protocols for data submission. A tiered approach accommodating different levels of complexity in data collection would enable a more streamlined and effective integration of research outcomes into regional and global databases, supporting international initiatives such as Regional Seas Convention groups and United Nations' Sustainable Development Goal databases. Overall, given the global scale of plastic pollution, future research should prioritise highly impacted and vulnerable areas. This targeted approach would ensure that efforts are concentrated where the ecological consequences are most severe, contributing to effective conservation and management strategies.

2.6 Conclusions

Rivers, fundamental to diverse ecosystems and invaluable for providing habitats and ecosystem services, play a pivotal role in understanding the global plastic mass balance equation (Everard and Moggridge, 2012; Harris et al., 2021a; Yeakley et al., 2016). Despite the conspicuous presence of macroplastics in certain rivers, large knowledge gaps persist, hindering our understanding of the potential impacts of plastic

pollution on these critical habitats (Blettler et al., 2018; van Emmerik and Schwarz, 2020). Addressing these gaps calls for comprehensive research, innovative strategies, and international collaboration to ultimately help assess the risk and mitigate the impacts concerning the escalating presence of plastic pollution in rivers.

Plastics potentially leach toxic, persistent, and bioaccumulative chemical substances and compounds, which are either intentionally added for functionality or inadvertently introduced substances resulting from production processes or environmental absorption (Groh et al., 2021, 2019b; UNEP and BRS Secretariat, 2023). It is subsequently of concern that microorganisms on plastic debris can facilitate chemical mobility, potentially increasing the toxic risk and endangering environmental health and food security (Mincer et al., 2019; Seltenrich, 2015), emphasising the need for a thorough understanding of its environmental impact.

Ultimately, building comparable datasets on plastic-biota interactions relevant to local regional ecological and anthropogenic settings is urgently needed. This study starts to bridge this gap by introducing a straightforward and cost-effective experimental design, to enable easy exploration of the potential impacts of floating macroplastics on the productivity, abundance and functional diversity of naturally occurring plankton communities. This approach provides an experimental system capable of delivering comparable data on plastic-biota interactions, focusing on the colonisation of plastic and influence on in-situ plankton communities, that can be deployed in remote settings with minimal equipment and training required.

While the experimental design provides a basic understanding of the impact of plastic on estuarine microcosms, future considerations and improvements proposed would refine and amplify the relevance and impact of these findings in the broader context of plastic pollution research. Overall, this study contributes the framework of a viable methodology to investigate microbial communities associated with floating macrolitter but also raises essential questions for future research and policy considerations in the broader context of monitoring ecosystem health and sustainability. The study's findings and limitations provide a basis for continued exploration in the emerging field of plastisphere research, emphasising the unique influence of biomes on plastic, their potential to alter ecosystem communities, dynamics, and productivity.

Chapter 3: Exploring nitrogen isotope ratios in mesopredatory sharks as a potential indicator of anthropogenic pollution

3.1 Abstract

Urbanisation has contributed to the degradation and physical alteration of coastal habitats, with chemical, nutrient, and plastic pollutants adding to anthropogenic pressures on marine communities. Understanding ecosystem-level impacts of anthropogenic pollutants is challenging due to the diverse nature of complex systems. In this preliminary investigation, ecological parameters were used to examine if a nitrogen isotope ratio ($\delta^{15}\text{N}$) response to anthropogenic pollution associated with coastal urbanisation could be detected in mesopredatory shark species. Utilising the Chondrichthyes Stable Isotope Database Project (CSIDP), the $\delta^{15}\text{N}$ signatures of 1,423 individual samples of species categorised as coastal mesopredatory sharks were examined. Generalised linear mixed effects models were applied to explore the $\delta^{15}\text{N}$ values from shark muscle tissue in relation to their potential pollution exposure accounting for species, total length of individuals, location, and capture depth, where possible. $\delta^{15}\text{N}$ values were evaluated in individuals caught in areas near urban centres compared to those from relatively rural locations. Enrichment of $\delta^{15}\text{N}$ in shark tissues likely indicates human pollution effects on nitrogen isotope baselines. Subsequently, these results support further investigation into the potential of mesopredatory sharks as bioindicators for anthropogenic pollution in coastal environments. Future research efforts should prioritise investigating more urbanised areas in relation to mesopredatory shark isotope data, incorporate additional environmental variables, and explore the potential mechanisms and sources associated with the observed nitrogen accumulation in shark tissues observed in this study.

3.2 Introduction

3.2.1 Environmental monitoring and anthropogenic pollution

Research on the cumulative impacts of anthropogenic-induced pollution on marine ecosystems remains limited, primarily due to the complexity of disentangling the effects of individual stressors and their interactions across intricate food webs (Todd et al., 2019). Pollution disrupts species distribution, community structures, and ecological functions in aquatic systems, with pronounced impacts in regions where pollutants accumulate, such as estuaries and semi-enclosed bays near urban and industrialised centres (Rangel et al., 2022a; Zhou et al., 2022). These areas often serve as hotspots for pollutants, including heavy metals, persistent organic pollutants (POPs), (micro)plastics, and nutrient runoffs, which can have cascading effects on marine biodiversity and ecosystem services (Halpern et al., 2008; Rochman et al., 2013)

Higher trophic level species, such as sharks, large teleosts, and marine mammals, are particularly vulnerable to these stressors due to their position in the food web and capacity to bioaccumulate and biomagnify contaminants (Fisk et al., 2001; Jepson et al., 2016). Despite their ecological significance and potential as indicators of ecosystem health, the impact of pollution on these species remain poorly understood compared to other anthropogenic pressures, such as fishing (Afonso and Fidelis, 2023; Dulvy et al., 2014). These knowledge gaps are further exacerbated by the challenges of studying wide-ranging species in dynamic environments, coupled with a global lack of resources and global capacity for long-term monitoring (Almroth et al., 2022; Larsen and Tararas, 2024). However, understanding these impacts are critical for addressing broader ecosystem health and resilience in the face of climate change, pollution, and biodiversity loss.

Stable Isotope Analysis (SIA) is a widely used tool in ecological research, offering valuable insights into nutrient cycling, species' foraging behaviours, and their roles within food webs. Initial studies have explored measuring nitrogen stable isotope values ($\delta^{15}\text{N}$) as an indicator of anthropogenic pollution in higher trophic level species,

including coastal fish and sharks. However, these studies have generally focused on one or two species at a limited number of sites (Prado et al., 2020; Rangel et al., 2022b).

This study aimed to investigate whether mesopredatory sharks serve as indicators of anthropogenic pollution-associated isotope markers through the food web. To achieve this, a global database of shark isotope data was analysed, incorporating samples from 18 species across 18 locations.

3.2.2 Stable isotope analysis in marine ecology research

Dissolved organic matter (DOM), a critical component of biogeochemical cycles, plays a significant role in driving isotopic variations in marine systems. DOM originates from primary production, terrestrial sources, and anthropogenic inputs, contributing to spatial isotope variations (Griffith and Raymond, 2011; Hansell and Carlson, 2014; Krause-Jensen et al., 2012; Opsahl and Benner, 1997).

The isotopic composition of animal tissues reflects the DOM that supports primary production (Davies et al., 2014; Sutton et al., 2018; Trueman and St John Glew, 2019). This includes carbon (C) and nitrogen (N), which are essential for quantifying the trophic niche width of species and characterising predator resource use and trophic levels (e.g., mesopredator, top predator (Layman et al., 2007; Shipley et al., 2017)).

Spatial models of isotopic ratios, or “isoscapes”, have been instrumental in understanding connections between systems, migrations of species, trophic dynamics, community ecology, population level-behaviours, and biogeochemical processes (Graham et al., 2010; McCauley et al., 2014; Reid et al., 2016; St John Glew et al., 2021). These isotopic signatures also provide a powerful means to detect ecosystem changes caused by pollution, climate change, or invasive species, by offering life history information that is otherwise difficult or impossible to obtain (Burton and Koch, 1999; Hobson, 1999; Trueman et al., 2012; Vander Zanden et al., 1999).

SIA is particularly effective for investigating these changes due to its cost efficiency and versatility. Compared to conventional diet-based methodologies, isotope approaches include non-lethal sampling of individuals (Hussey et al., 2012). Additionally, developing

stable isotope tracers to study pollutant impacts aligns with the United Nations Sustainable Development Goal (SDG) indicator 14.1.1, which focuses on monitoring coastal eutrophication parameters, including total nitrogen and chlorophyll-a concentrations (UNEP, 2021b). Expanding these monitoring parameters to an isotopic level would enable a more comprehensive understanding of ecosystem dynamics at both local and global scales.

3.2.3 Stable nitrogen isotope ratios

Stable nitrogen isotope ratio ($\delta^{15}\text{N}$) values in body proteins are primarily diet-derived and consistent across individuals, making them a valuable measure for assessing trophic dynamics and nitrogen assimilation (Adams and Sterner, 2000; Parker et al., 2005). These isotopic values are influenced by nitrogenous waste excretion, as organisms preferentially excrete the lighter isotope (^{14}N) at higher trophic levels (Fisk et al., 2002; Johnson et al., 1997). As nitrogen is metabolised, trophic-level enrichment occurs, with $\delta^{15}\text{N}$ values typically increasing by 2.0-3.4‰ per trophic step (Estrada et al., 2003; Post, 2002). The most prevalent application of $\delta^{15}\text{N}$ in marine isotope ecology is determining relative or absolute trophic positions (Shipley et al., 2017).

Although this predictable enrichment makes $\delta^{15}\text{N}$ a widely used proxy for trophic position, variations in diet-tissue fractionation ($\Delta^{15}\text{N}$), seasonal isotopic shifts, and the effects of lipids can introduce uncertainty (Hussey et al., 2010; Richert et al., 2015). Additionally, elevated concentrations of urea in chondrichthyan tissues are thought to have a deleterious effect on $\delta^{15}\text{N}$, resulting in a trophic shift of approximately 30-50% (Carlisle et al., 2017; Hussey et al., 2012). Spatially mapping gradients of $\delta^{15}\text{N}$ in marine ecosystems is also challenging due to the complexity of N cycling processes, including fixation and denitrification, which vary with factors such as plankton composition, bacterial activity, and water mass properties (DeNiro, 1987; Descolas-Gros and Fontugne, 1990; Granger et al., 2008).

Studies suggest that species inhabiting greater depths typically demonstrate higher $\delta^{15}\text{N}$ (Shipley et al., 2017), however, deep-sea chondrichthyans have also been observed to occupy similar trophic levels to coastal and pelagic species (Musick and Cotton, 2015; Pethybridge et al., 2012). In theory, low-productivity food webs may lead

to significant niche overlap among top predators due to increased competition driven by limited prey availability (Shipley et al., 2017).

Despite these complexities, the observable trends, similarities and dissimilarities of $\delta^{15}\text{N}$ serves as a valuable tool for examining nutrient flow and spatial distributions within food webs. However, its interpretation as a sole indicator of trophic dynamics requires caution (Fisk et al., 2002). A summary of typical $\delta^{15}\text{N}$ ranges across upwelling and oligotrophic regions in marine ecosystems and trophic levels is provided in Figure 3.1.

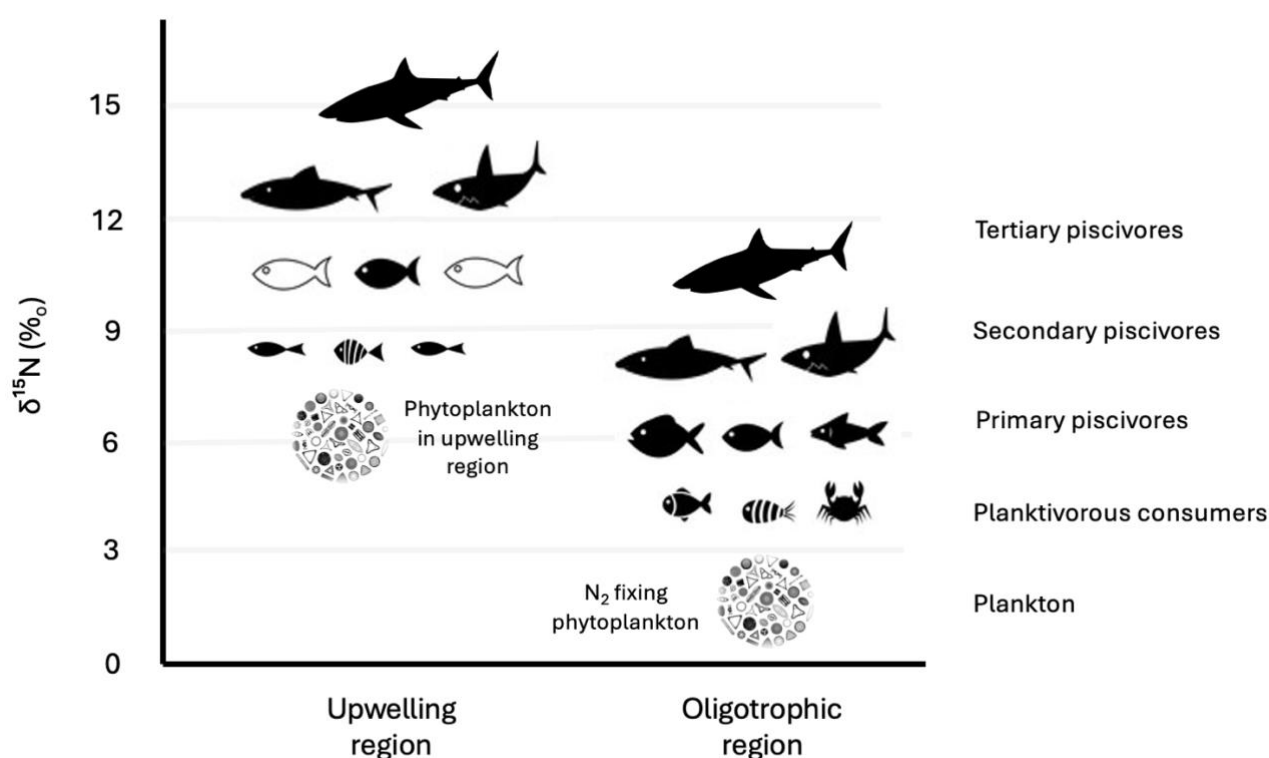


Figure 3.1: Summary of typical $\delta^{15}\text{N}$ ranges across upwelling and oligotrophic ecosystems and trophic levels (image adapted from (Estrada et al., 2005, 2003; Hussey et al., 2015; Richert et al., 2015)).

Pelagic predators in nutrient-rich, open ocean systems often exhibit higher $\delta^{15}\text{N}$ values due to their consumption of high trophic-level prey (Borrell et al., 2011; Estrada et al., 2003). In contrast, coastal and estuarine predators have generally been found exhibit lower $\delta^{15}\text{N}$ values, reflecting diets based on lower-trophic-level prey, such as crustaceans and inshore fishes, which feed in isotopically lighter benthic environments .

Similarly, reef-associated species often show moderate $\delta^{15}\text{N}$ values, indicative of diverse diets and efficient trophic transfer within reef ecosystems (Kiszka et al., 2015; Papastamatiou et al., 2010). Isotopic enrichment with increasing body size has also been reported for a wide range of species (Dalponti et al., 2018; Jennings et al., 2008, 2002). However, exceptions exist, particularly in large planktivorous species and predators that consume planktivorous or herbivorous prey (Estrada et al., 2003; Heithaus et al., 2013). These patterns underscore the influence of habitat-specific nitrogen baselines and prey composition on $\delta^{15}\text{N}$ values, highlighting the need for careful, context-dependent interpretation.

3.2.4 Stable nitrogen isotope ratios and anthropogenic impacts

In addition to its role as a trophic marker, $\delta^{15}\text{N}$ has been linked to anthropogenic influences associated with urbanisation and industrialisation (Cabana and Rasmussen, 1994; McClelland et al., 1997). Anthropogenic nitrogen inputs, such as those from domestic sewage effluent and agriculture runoff, are often enriched in the heavy isotope of nitrogen (^{15}N) relative to marine nitrate sources (Harrington et al., 1998; Savage, 2005). This enrichment can elevate $\delta^{15}\text{N}$ values in marine organisms, providing a tracer for the incorporation of anthropogenic nitrogen into food webs (Mancinelli and Vizzini, 2015; McClelland and Valiela, 1998).

Certain species, such as bivalves, have been identified as effective indicator species for anthropogenic-induced eutrophication (Puccinelli et al., 2016; Raikow and Hamilton, 2001; Thibault et al., 2020). These findings suggest that $\delta^{15}\text{N}$ can be a valuable tool for monitoring the ecological impacts of urbanisation and pollution, particularly in coastal environments where nutrient inputs are high. Moreover, integration $\delta^{15}\text{N}$ data with other ecological indicators can enhance our understanding of how anthropogenic activities reshape marine food webs.

3.2.5 Defining mesopredatory sharks

Apex predators have been defined as species occupying the top trophic position within a community, known for exerting strong effects on trophic dynamics and diversity (Ritchie and Johnson, 2009). Comparatively, mesopredators occupy intermediate trophic positions, exerting more diffuse predation pressure within food webs. These species often exploit a broader range of food resources, exhibit less specialisation, and exert a weaker influence on the behaviour of other species compared to apex predators (Heupel et al., 2014). Their intermediate trophic position enables them to act as both predators and prey, linking lower and upper trophic levels.

From an isotopic perspective, mesopredators' relatively smaller size and limited spatial distribution make them more reflective local food web structures and nutrient cycling processes (Richert et al., 2015). This localised isotopic signature is particularly useful in ecological studies, as it provides insights into regional food web dynamics and environmental influences. Additionally, intra-species variations often masks inter-species differences, which could help efforts to generalise trophic interactions across mesopredatory species (Kiszka et al., 2015).

The classification of species as an apex or mesopredator is highly context dependent, influenced by habitat, life stage, and ecological interactions. For instance, a species may function as an apex predator in a resource-limited or predator-absent habitat but act as a mesopredator in more competitive ecosystems. This duality is evident in large shark species, which are often broadly categorised as 'apex' predators but may exhibit mesopredatory behaviours depending on their size, diet, and ecological context (Benavides et al., 2011; Heupel et al., 2014). For example, reef sharks are often assumed to be apex predators due to their large size and behaviour, however their functional role is similar to large predatory fishes, typically regarded as high trophic level mesopredators (Frisch et al., 2016).

Given these complexities, this study adopted a size-based criterion for mesopredatory sharks, focusing on mature individuals less than 300 cm in total length. This threshold aligns with established research highlighting the ecological characteristics of mesopredators (Heupel et al., 2014). By targeting this size class, the study aimed to

capture the localised isotopic signatures of mesopredatory sharks while maintaining consistency with broader trophic frameworks.

3.3 Methods

The original aim for this study was to investigate isotopic data of higher and lower trophic level species in Manado, Indonesia, between ecologically similar sites with contrasting levels of floating macroplastic pollution. The objectives were to evaluate feeding ecologies and movement patterns of coastal predators to understand if plastic loading has the potential to food web dynamics. Due to COVID-19 we were not able to carry out the fieldwork that had been planned.

3.3.1 The Chondrichthyes stable isotope database project (CSISP)

The Chondrichthyes stable isotope database project (CSIDP) was established in 2018 as a collaborative project to share data from 54 publications (2007-2018) and 7 unpublished datasets (Bird et al., 2018). The CSIDP contains measurements of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in muscle tissue for over 7,000 individual sharks from 114 species and has been used to investigate the global foraging habits of sharks (Bird et al., 2018). All procedures involving the handling and analysis of biological samples related to the CSIDP were conducted in accordance with relevant ethical guidelines and regulations, ensuring the responsible and ethical use of marine resources for scientific inquiry.

In most cases CSIDP entries are accompanied by metadata including total length, capture area and capture depth, although some samples are taken from a landing dock. Not all studies applied lipid extraction or applied corrections to account for lipid in the tissues. Isotopic compositions are expressed in δ values (parts per thousand differences from a standard or per mil (‰)) and are calculated using the equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000$$

where $X = {}^{13}\text{C}$ or ${}^{15}\text{N}$, $R = \text{ratio of } {}^{13}\text{C}/{}^{12}\text{C}, {}^{15}\text{N}/{}^{14}\text{N}$, and the standards are Vienna Pee Dee Belemnite limestone (V-PDB) for carbon and air for nitrogen (Newsome et al., 2007).

In this study, targeted exclusion criteria were implemented to restrict analysis to coastal mesopredatory shark populations. Specifically, pelagic species, individuals exceeding 300 cm in length, and individuals known to be juvenile were excluded, recognising mother-embryo isotope bias (Olin et al., 2011). Samples with a known capture depth of >200 m were also excluded, as they are not associated with coastal ecosystems and rely on different marine resources (Bird et al., 2018). Considering fractionation differences between different tissues, only muscle data was explored. This approach yielded a dataset comprising 1,423 individuals (51% female, 36% male, 13% unknown) from 18 species, each with a robust sample size ($N > 30$) (Table 3.1). The average TL was 98.71 cm [$SD = 22.46$] and average depth was 47.19 m [$SD = 66.77$], where recorded.

Table 3.1: Mesopredatory samples obtained from the CSIDP database detailing species, feeding habitats and prey based on information available on FishBase, sample N, sample location(s), average total length (TL cm), and average capture depth (m).

Species	Feeding habitat	Typical prey	Total sample N	Sample location(s) and sample N	Average TL (cm)	Average depth (m)
<i>Carcharhinus amblyrhynchos</i>	Coatsal-pelagic and reef-associated	Bony fishes, cephalopods, crustaceans	32	Great Barrier Reef	115 ± 32	25
<i>Carcharhinus brachyurus</i>	Coatsal-pelagic and reef-associated	Bony fishes, cephalopods, crustaceans	87	Gulf of St. Vincent (N = 33), Spencer Gulf (N = 44)	118 ± 29	n/a
<i>Carcharhinus falciformis</i>	Pelagic and reef-associated	Bony fishes, cephalopods, crustaceans	80	CE Pacific (N = 46), Florida (N = 1), Mayotte (N = 30), Nosy Hao (N = 3)	117.39 ± 37	n/a
<i>Carcharhinus limbatus</i>	Coatsal-pelagic and reef-associated	Bony fishes, cephalopods, crustaceans	47	Galveston (N = 36), KZN Nets (N = 11)	139 ± 30	30
<i>Carcharhinus perezii</i>	Coatsal-pelagic and reef-associated	Bony fishes, cephalopods, crustaceans	31	New Providence	146 ± 34	24 ± 9
<i>Carcharhinus plumbeus</i>	Coatsal-pelagic and benthopelagic	Bony fishes, cephalopods, crustaceans	79	St. Helena	77 ± 21	n/a
<i>Galeocerdo cuvier</i>	Coatsal-pelagic, benthopelagic and reef-associated	Bony fishes, cephalopods, crustaceans, marine mammals, sea turtles, seabirds	36	Great Barrier Reef (N = 1), KZN Nets (N = 2), Queensland (N = 33)	149 ± 27	28 ± 3
<i>Loxodon macrorhinus</i>	Benthopelagic	Small bony fishes, cephalopods, crustaceans	83	Nosy Hao	84 ± 10	n/a
<i>Rhizoprionodon taylori</i>	Benthopelagic	Small bony fishes, cephalopods, crustaceans	159	Queensland	66 ± 7	10 ± 1
<i>Rhizoprionodon terraenovae</i>	Benthopelagic	Bony fishes, cephalopods, crustaceans	50	Galveston	89 ± 13	n/a
<i>Scoliodon laticaudus</i>	Benthopelagic and estuarine	Small bony fishes, crustaceans, mollusks	165	Hong Kong	40 ± 14	40
<i>Scyliorhinus canicula</i>	Benthopelagic	Crustaceans, mollusks, small fishes	119	Balearic Island (N = 54), English Channel (N = 36), North Sea (N = 29)	42 ± 12	84 ± 53
<i>Sphyrna lewini</i>	Coatsal-pelagic and benthopelagic	Bony fishes, cephalopods, crustaceans	95	CE Pacific (N = 6), Florida (N = 24), KZN Nets (N = 23), Nosy Hao (N = 38), Queensland (N = 4)	92 ± 40	30
<i>Sphyrna tiburo</i>	Benthopelagic and estuarine	Crustaceans, mollusks, small fishes	74	Florida (N = 24), Galveston (N = 50)	85 ± 26	n/a
<i>Sphyrna zygaena</i>	Pelagic and benthopelagic	Bony fishes, cephalopods, crustaceans	31	CE Pacific (N = 19), KZN Nets (N = 12)	147 ± 23	30
<i>Squalus acanthias</i>	Benthopelagic	Bony fishes, cephalopods, crustaceans	167	Aomori (N = 75), North Sea (N = 92)	89 ± 16	n/a
<i>Squalus megalops</i>	Benthopelagic	Bony fishes, cephalopods, crustaceans	57	Reunion Island	56 ± 13	n/a
<i>Triaenodon obesus</i>	Coatsal-pelagic and reef-associated	Bony fishes, cephalopods, crustaceans	31	Great Barrier Reef	126 ± 19	25

3.3.2 Estimating population density

The investigation encompasses 18 distinct geographical locations (N > 30), 3 of which are categorised to have a high local population density (>1000 people per km²) (Figure 3.1).



Figure 3.2: Map illustrating count of shark muscle samples (circle size) from the Chondrichthyes Stable Isotope Database Project (CSIDP) by location and population density category (circle colour). Map generated using Tableau software.

Local population density was estimated by referencing the latest available census data from National Statistical Offices corresponding to each geographical location (Table 3.2). Population density was subsequently categorised into three density tiers based on population density (Dijkstra et al., 2021). Areas with a population density exceeding 1,500 people per km² were designated as an urban centre. Those with densities falling between 300 and 1500 people per km² were considered an urban cluster, and regions outside urban clusters were classified as rural areas (Table 3.2).

Table 3.3: Population density estimates for categorisation into three distinct levels of urbanisation (Dijkstra et al., 2021), population data sourced from online metadata based on census data (<https://www.citypopulation.de/en/>).

Sample location	Population density (people/km ²)	Date	Population density category
Aomori	334	2020	Urban cluster
Balearic Island	243	2020	Rural
CE Pacific (uninhabited)	0		Rural

English Channel	536	2019	Urban cluster
Florida (Bay County)	97	2023	Rural
Galveston	500	2022	Urban cluster
Great Barrier Reef	3	2019	Rural
Gulf St. Vincent	268	2021	Rural
Hong Kong	6,668	2021	Urban centre
KZN Nets (KwaZulu-Natal)	132	2022	Rural
Mayotte	860	2020	Urban cluster
New Providence	1,532	2022	Urban centre
North Sea	250	2009	Urban cluster
Nosy Hao	342	2018	Urban cluster
Queensland	3	2021	Rural
Reunion Island	393	2023	Urban cluster
Spencer Gulf	2	2021	Rural
St Helena (Beaufort County)	133	2023	Rural

3.3.3 Corrected $\delta^{15}\text{Nc}$ values

Corrections to baseline isotope values can reveal patterns that may otherwise remain elusive (Matich et al., 2021). Baseline variation in $\delta^{15}\text{N}$ values was accounted for by referencing measured values to a mechanistic model predicting $\delta^{15}\text{N}$ values in plankton (Somes et al., 2010). The Somes et al. (2010) isoscape model predicts $\delta^{15}\text{N}$ values across various components of the marine ecosystem, including nitrate (NO_3^-), phytoplankton, zooplankton, and detritus variables. Additionally, it accounts for isotope effects associated with algal NO_3^- uptake, nitrogen fixation, water column denitrification, and zooplankton excretion, along with the removal of NO_3^- by sedimentary denitrification. To establish a corrected $\delta^{15}\text{N}$ value ($\delta^{15}\text{Nc}$) for the sampled areas, baseline values from this model were utilised to enable the removal of any background signal attributable to local biogeochemical processes from the $\delta^{15}\text{N}$ values observed in this study. This procedure ensured that the isotope signatures more accurately reflect the specific contributions from the diet and habitat of the sharks under study, independent of broader environmental influences. It should be noted, due to the fast nitrogen turnover of plankton, baseline variation in $\delta^{15}\text{N}$ is difficult to resolve and results in large temporal variability (Cabana and Rasmussen, 1996). To underline, these are model estimates based on limited sampled data and this model assumes that

anthropogenic induced baseline $\delta^{15}\text{N}$ values do not already capture a high anthropogenic load.

3.3.4 Statistical analysis

This study investigated variance in stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) among 18 mesopredatory elasmobranch species sampled across 18 geographical locations. Parametric statistics were performed to explore species and location groups. Normality was tested using Shapiro-Wilks test and a Levene's test was used to test for homogeneity of variances. Significant Welch's ANOVA results were followed by a post-hoc Games-Howell test to evaluate the variables of interest.

To test for relationships between $\delta^{15}\text{N}$ values and potential predictor variables, General Linear Mixed Models (GLMM) were utilised. Models were fitted with different combinations of the following variables: level of anthropogenic impact categorised by population density (urban centre/urban cluster/rural), depth of capture (m), total length (TLcm), and location. The GLMM framework allows for incorporation of both fixed effects, representing systematic factors of interest (TLcm, depth (m), and population), as well as random effects, capturing additional sources of expected variance (species and location). In most cases, species and location terms covary with study ID, so study ID was not included as a specific random effect. Of the predictor variables tested, total length and depth were the only continuous variables, while the population terms were categorical and subsequently reported separately to provide clear insights into how each category influences the outcome.

The GLMMs were fitted using the lme4 package (Wang et al., 2022) within the R programming environment. Model performance and validity were assessed through Akaike Information Criterion (AIC) values, which provide a measure of the model's fit. Lower AIC values indicate a better balance between goodness of fit and complexity, as AIC penalises model complexity to avoid overfitting. This approach allowed for assessment of the extent of variance in $\delta^{15}\text{N}$ isotope values explained by variation in the predictor variables of interest, as well as the examination of the variance associated with random effects.

Effect estimates for the best fitting models were extracted and displayed graphically. Additionally, Principal Component Analysis (PCA) was employed to assess the variance associated with $\delta^{15}\text{Nc}$, total length (cm) and depth (m), aiming to visualise the similarities and dissimilarities among samples, categorised by local population density. The eigenvalues from PCA measure the amount of variation retained by each principal component. An eigenvalue > 1 indicates that principal components (PCs) account for more variance than accounted by one of the original variables in standardised data (Kaiser 1961). This is commonly used as a cut-off point for which PCs are retained but only holds true only when the data are standardised. Therefore, material estimates were transformed as follows:

$$x^i - \text{mean}(x)/\text{sd}(x)$$

Where mean (x) is the mean of x values, and sd(x) is the standard deviation (SD).

The eigenvalues determined the PCs subsequently plotted. Positive loadings indicate that high values of the variable are associated with high values of the component, while negative loadings indicate the opposite. The magnitude of the loading indicates the strength of the association. Vector arrows were also plotted to show the relationships between variables. All analyses were performed using R.

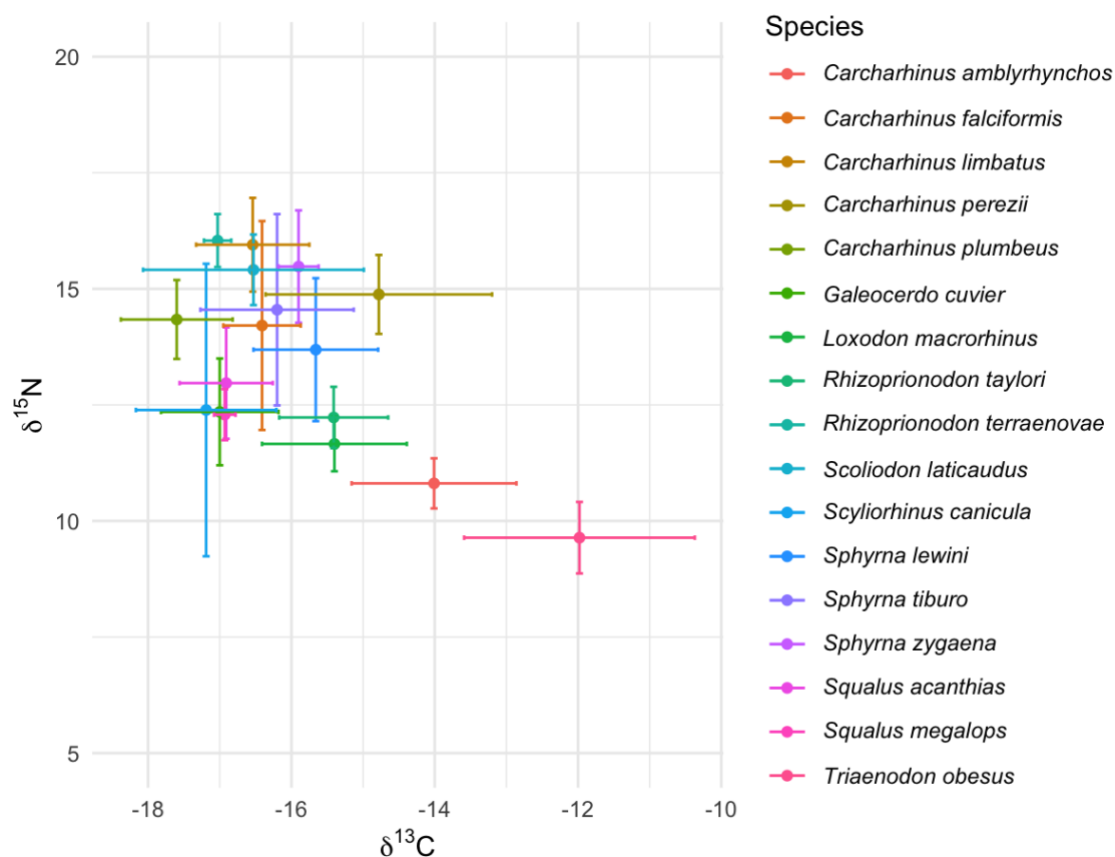
3.4 Results

3.4.1 Isotope values considering species and location groups

Half of the species sampled were captured from a single location (Table 3.2). The mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and $\delta^{13}\text{C}$ and $\delta^{15}\text{Nc}$ values (\pm SD) for each species are shown in Figure 3.2, detailed in Appendix II, Table 1. *Carcharhinus brachyurus* samples, all from the Gulf of St. Vincent, were missing $\delta^{13}\text{C}$ data. *Triaenodon obesus* samples, all from the Great Barrier Reef, reported the most positive mean $\delta^{13}\text{C}$ value (-11.98 ± 1.61 ‰), while *Carcharhinus plumbeus* samples, all from St. Helena Island in South Carolina, reported the most negative mean $\delta^{13}\text{C}$ value (-17.60 ± 0.78 ‰).

In assessment of nitrogen isotope values, *T.obesus* reported the lowest value for both mean $\delta^{15}\text{N}$ (9.64 ± 0.77 ‰) and mean $\delta^{15}\text{Nc}$ (7.02 ± 0.77 ‰). *Rhizoprionodon terraenovae* samples, all from Galveston in the Gulf of Mexico, reported the highest mean values for both $\delta^{15}\text{N}$ and $\delta^{15}\text{Nc}$ (16.04 ± 0.57 ‰ and 17.70 ± 0.57 ‰, respectively).

a.



b.

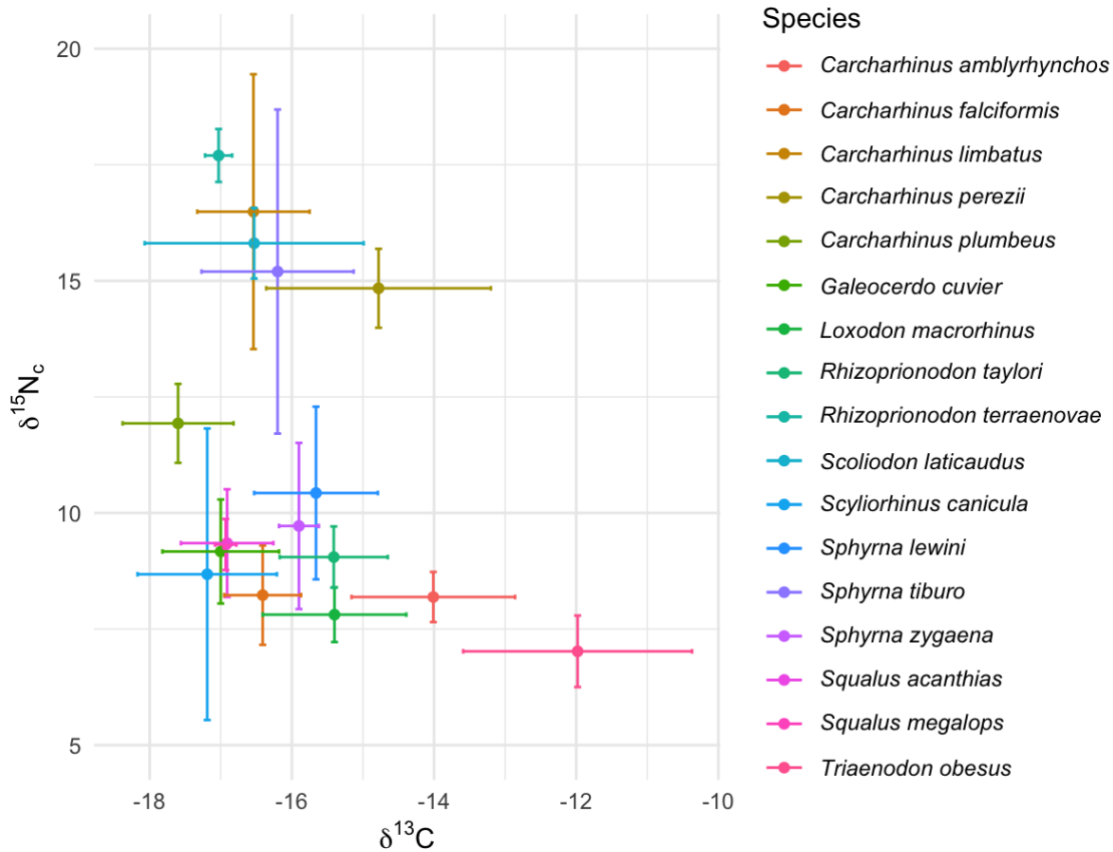


Figure 3.2: Scatterplot with mean and standard deviation for a) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and b) $\delta^{15}\text{Nc}$ and $\delta^{13}\text{C}$ values of shark species from the CSIDP. Each point represents the mean value of species ($N = >30$).

Considering $\delta^{15}\text{Nc}$ values, the Levene's test for homogeneity of variances failed ($F_{17, 1405} = 39.26$, $p < 0.01$). Subsequently, significant differences were found among species groups according to Welch's ANOVA results ($F_{17, 365} = 1299.22$, $p < 0.01$). The between-species variability for $\delta^{15}\text{Nc}$ (10.82 ± 3.49 ‰) was larger than the variability within most species' groups (Appendix II, Table 1). Species taken from a single location had a comparatively narrow $\delta^{15}\text{Nc}$ range [$SD = <1.00$ ‰] (Appendix II, Table 2). A pairwise comparison of species groups using a Games-Howell test is provided in Appendix II (Table 3). Notably, *R.terraenovae*, *Carcharhinus limbatus*, *Scoliodon laticaudus*, *Sphyrna tiburo*, and *Carcharhinus perezii* exhibit significantly high $\delta^{15}\text{Nc}$ values, averaging >14.00 ‰ (Figure 3.3).

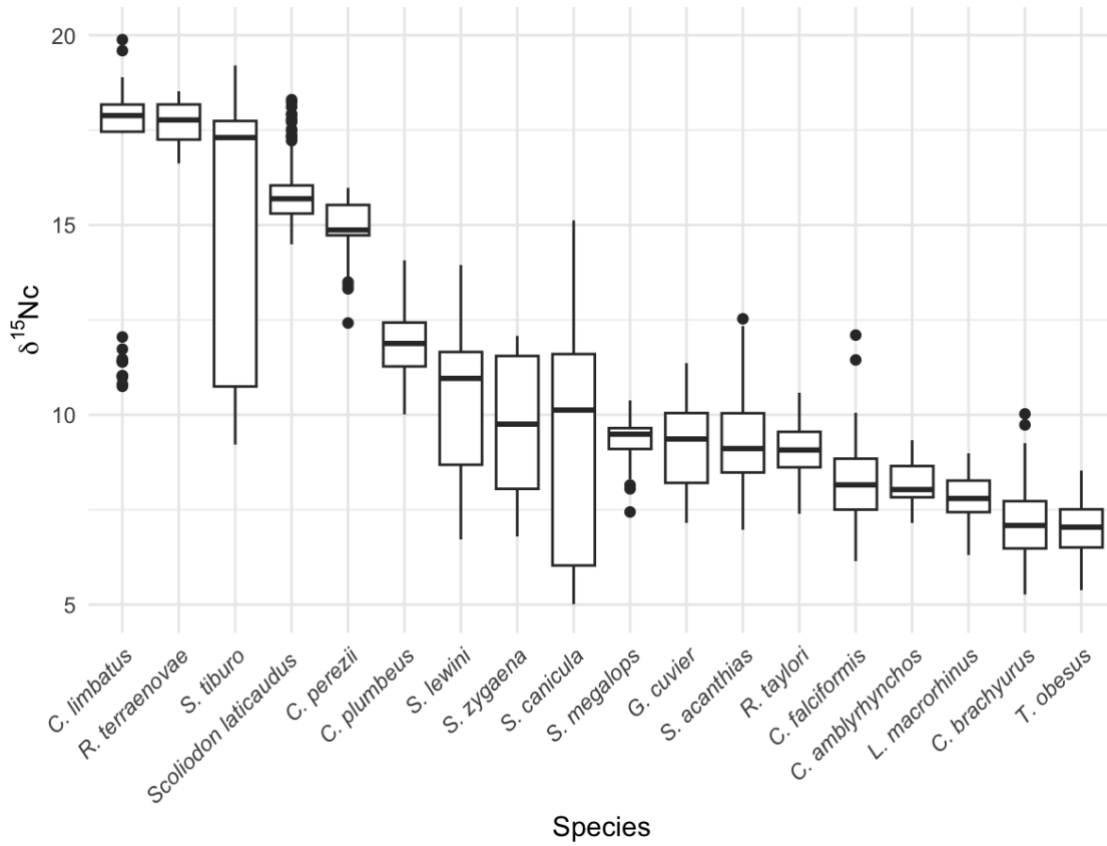


Figure 3.3: Box plots of $\delta^{15}\text{Nc}$ for mesopredatory shark samples grouped by species from the CSIDP (see table 3.2 for details regarding N of species and sample locations).

Considering sample locations, the Levene's test for homogeneity of variances failed ($F_{17, 1405} = 18.18$, $p < 0.01$) and significant differences were found among sample locations according to Welch's ANOVA results ($F_{17, 369} = 2031$, $p < 0.001$). The between-location variability of $\delta^{15}\text{Nc}$ (10.61 ± 3.26) was larger than the variability within locations (Appendix II, Table 2). Further exploration through a Games-Howell test is provided in Appendix II (Table 4). Notably, 3 areas (Galveston, Hong Kong, and New Providence) showed comparatively high $\delta^{15}\text{Nc}$ values, averaging >14.00 ‰ (Figure 3.4).

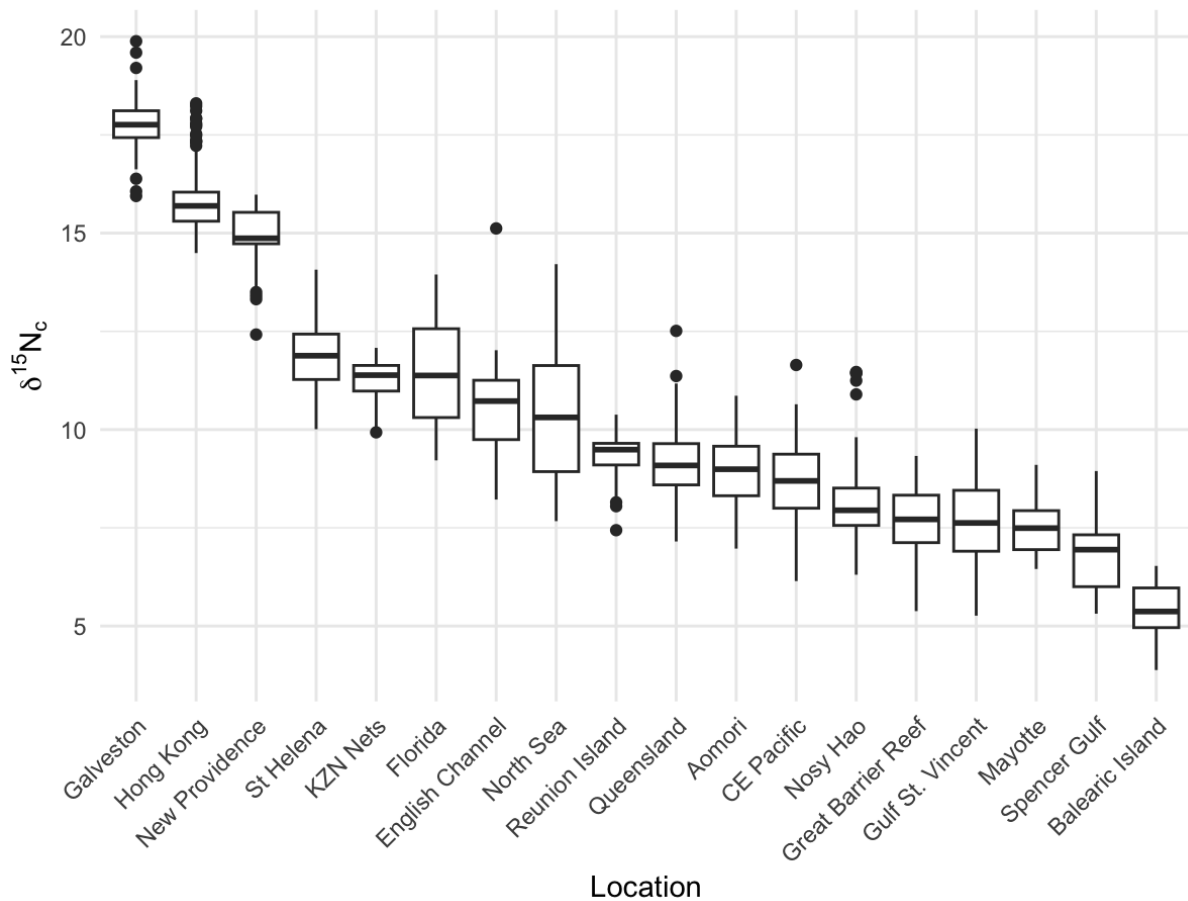


Figure 3.4: Box plots of $\delta^{15}\text{N}_c$ for mesopredatory shark samples from the CSIDP grouped by sample location.

3.4.2 Modelling variation in $\delta^{15}\text{N}_c$ values

Only 552 samples had associated depth data, which were mainly between 10-40m, while 1,400 samples provided length data. Significant negative and positive relationships between TLcm and $\delta^{15}\text{N}_c$ values were found for 9 of the species groups (Appendix II, Table 5). Only *Scyliorhinus canicula* samples from the English Channel, North Sea and Balearic Islands provided samples from a wider depth range (between 26-200 m). A statistically significant negative correlation between depth and $\delta^{15}\text{N}_c$ values were found for *S.canicula* samples ($F_{1,88} = 116.10, p < 0.01$).

A total of 15 GLMMs were fitted from the combination of 5 potential explanatory variables (ecological factors) to investigate their relationship with $\delta^{15}\text{N}_c$ values (Table 3.3). This process identified M9 as the optimal model, characterised by the lowest AIC

score (1298.17). The exclusion of depth to investigate a larger sample size highlighted M13 as the optimal model, however the AIC score is substantially higher.

Subsequently, model 9 (M9) and model 13 (M13) underwent fitting using restricted maximum likelihood (REML) estimation, achieving convergence at 1280.8 and 3593.9, respectively (Table 3.4). Model performance tests are provided in Appendix II (Figure 1-3).

Table 3.3: Results from GLMMs fitted using mesopredatory elasmobranch data from the CSIDP, ranked by AIC for $\delta^{15}\text{Nc}$ values. Explanatory variables included in the models: Species, location, total length (TL cm), depth (m) and population density (categorical variable). The best fitting models with and without depth data are highlighted in bold.

Model		df	AIC
#	LMM		
1	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Population} + \text{Depth} + (1 \text{Species})$	7	1302.629
2	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Population} + \text{Depth} + (1 \text{Location})$	7	1329.807
3	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Population} + (1 \text{Location})$	6	3888.969
4	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Population} + (1 \text{Species})$	6	5066.348
5	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Depth} + (1 \text{Location})$	5	1340.457
6	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Depth} + (1 \text{Species})$	5	1612.269
7	$\delta^{15}\text{Nc} \sim \text{Population} + \text{Depth} + (1 \text{Location})$	6	1330.430
8	$\delta^{15}\text{Nc} \sim \text{Population} + \text{Depth} + (1 \text{Species})$	6	1305.631
9	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Population} + \text{Depth} + (1 \text{Species}) + (1 \text{Location})$	8	1296.807
10	$\delta^{15}\text{Nc} \sim \text{Population} + (1 \text{Species}) + (1 \text{Location})$	6	3651.506
11	$\delta^{15}\text{Nc} \sim \text{TL cm} + (1 \text{Species}) + (1 \text{Location})$	5	3617.202
12	$\delta^{15}\text{Nc} \sim \text{Depth} + (1 \text{Species}) + (1 \text{Location})$	5	1309.59
13	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Population} + (1 \text{Species}) + (1 \text{Location})$	7	3607.807
14	$\delta^{15}\text{Nc} \sim \text{TL cm} + \text{Depth} + (1 \text{Species}) + (1 \text{Location})$	6	1308.61
15	$\delta^{15}\text{Nc} \sim \text{Population} + \text{Depth} + (1 \text{Species}) + (1 \text{Location})$	7	3651.506

In assessing the fixed effects for M9, the reference intercept was estimated at 8.34 ‰ [SE = 0.97] (Table 3.4). The estimates for urban clusters and urban centres both reported increased $\delta^{15}\text{Nc}$ values compared to the reference intercept, with an estimate of 6.89 ‰ $\delta^{15}\text{Nc}$ ([SE = 1.66], $t = 4.15$) reported for samples in locations categorised as

urban centres. TL and depth yielded weak coefficients, with TL indicating a small positive effect and depth a small negative one (Table 3.4).

In assessing the fixed effects for M13, excluding depth as a variable and increasing the sample size, the reference intercept was estimated at 8.35 ± 1.24 ‰ (Table 3.4). The intercept estimates for urban clusters (1.78 ‰ $\delta^{15}\text{Nc}$ ([$SE = 1.51$], $t = 1.81$) and urban centres (6.66 ‰ $\delta^{15}\text{Nc}$ ([$SE = 2.38$], $t = 2.79$) reported slightly smaller positive effects on $\delta^{15}\text{Nc}$ values compared to M9, although the coefficients reported more robust t -values (Table 3.4). The estimate for TL was higher for M13 than M9, with a more robust t -value (Table 3.4). Plots of the fixed effects for M9 and M13 are provided in Figure 3.5.

The random effects component of both M9 and M13 accounted for variability at species and location levels (Table 3.4). For M9, the residual variance explained by the intercept for species and location were 0.41 ± 0.64 ‰ and 3.10 ± 2.13 ‰, respectively. In comparison to M9, the residual variance of the random effects in M13 increased with 8 more species included in the model (0.70 ± 0.83 ‰) and 11 more locations (8.61 ± 2.93 ‰). Plots for the random effects of species and location for M9 and M13 are provided in Figure 3.6 and Figure 3.7.

Table 3.4: Results from extraction of fixed and random effects from GLMM of M9 (N = 552; Species N = 10, Location N = 7) and M13 (N = 1400; Species N = 18, Location N = 18).

M9: $\delta^{15}\text{Nc} \sim \text{Total length (cm)} + \text{Population (category)} + \text{Depth (m)} + (1|\text{Species}) + (1|\text{Location})$

Scaled Residuals				
Min	1Q	Median	3Q	Max
-3.31	-0.61	-0.06	0.58	6.18
Random Effects				
Groups	Name	Variance	Std.Dev.	
Species	(Intercept)	0.41	0.64	
Location	(Intercept)	3.10	1.82	
Residual		0.53	0.73	
Fixed Effects				
	Estimate	Std. Error	t value	
(Intercept)	8.34	0.97	8.62	
Urban centre	6.89	1.66	4.15	

Urban cluster	2.62	2.10	1.25
TLcm	0.002	0.002	1.32
Depth	-0.003	0.002	-1.47

M13: $\delta^{15}\text{Nc} \sim \text{TLcm} + \text{Population} + (1|\text{Species}) + (1|\text{Location})$

Scaled residuals:

Min	1Q	Median	3Q	Max
-3.12	-0.67	-0.02	0.61	5.54

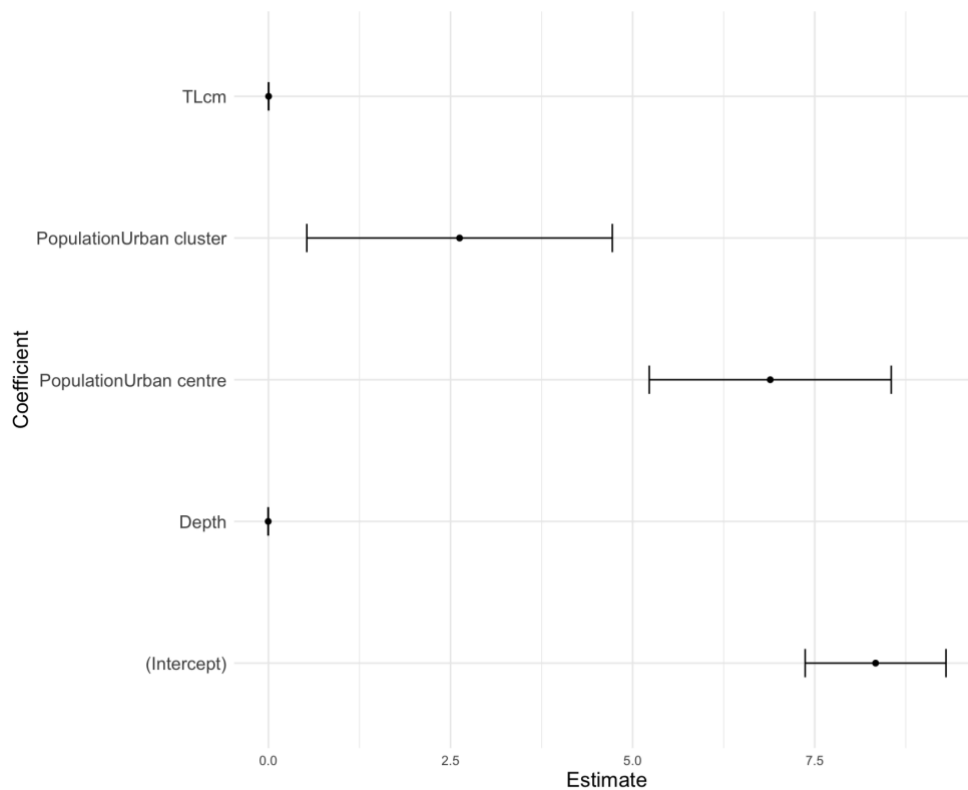
Random effects:

Groups	Name	Variance	Std.Dev.
Species	(Intercept)	0.70	0.83
Location	(Intercept)	8.61	2.93
Residual		0.68	0.83

Fixed effects

	Estimate	Std. Error	t value
(Intercept)	8.35	1.24	12.93
TLcm	0.004	0.001	3.15
Urban centre	6.66	2.38	2.79
Urban cluster	1.78	1.51	1.81

a.



b.

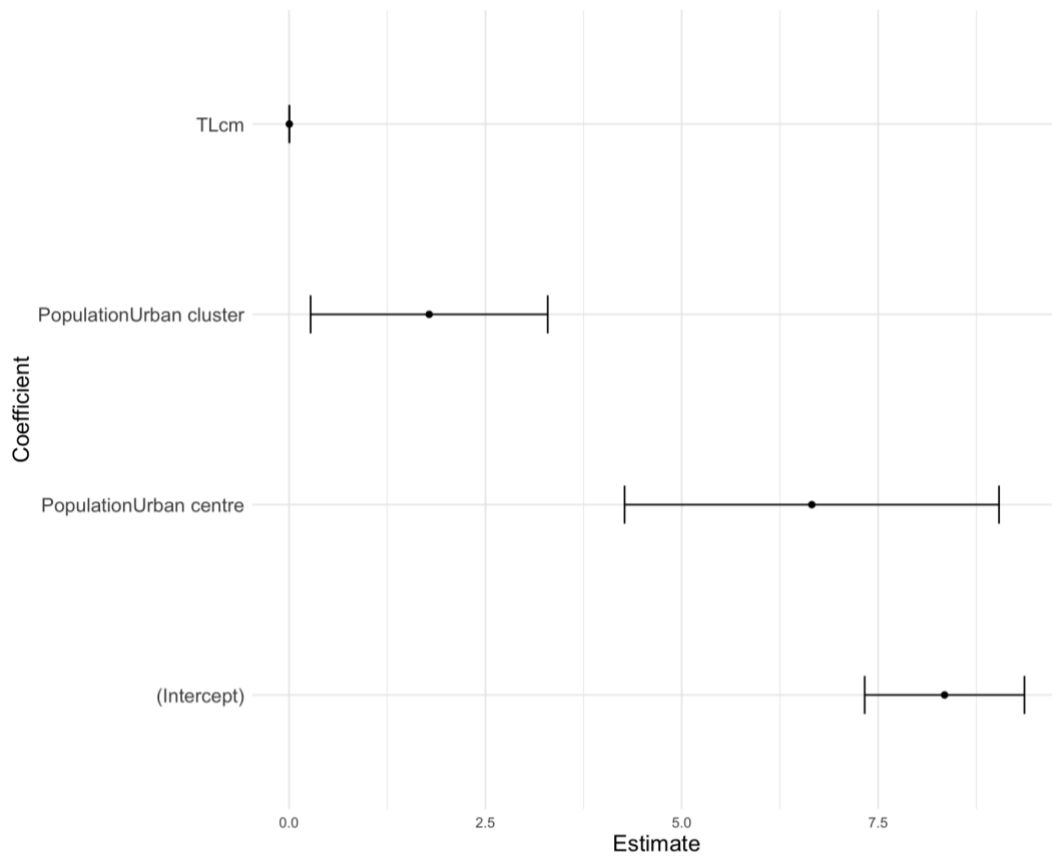
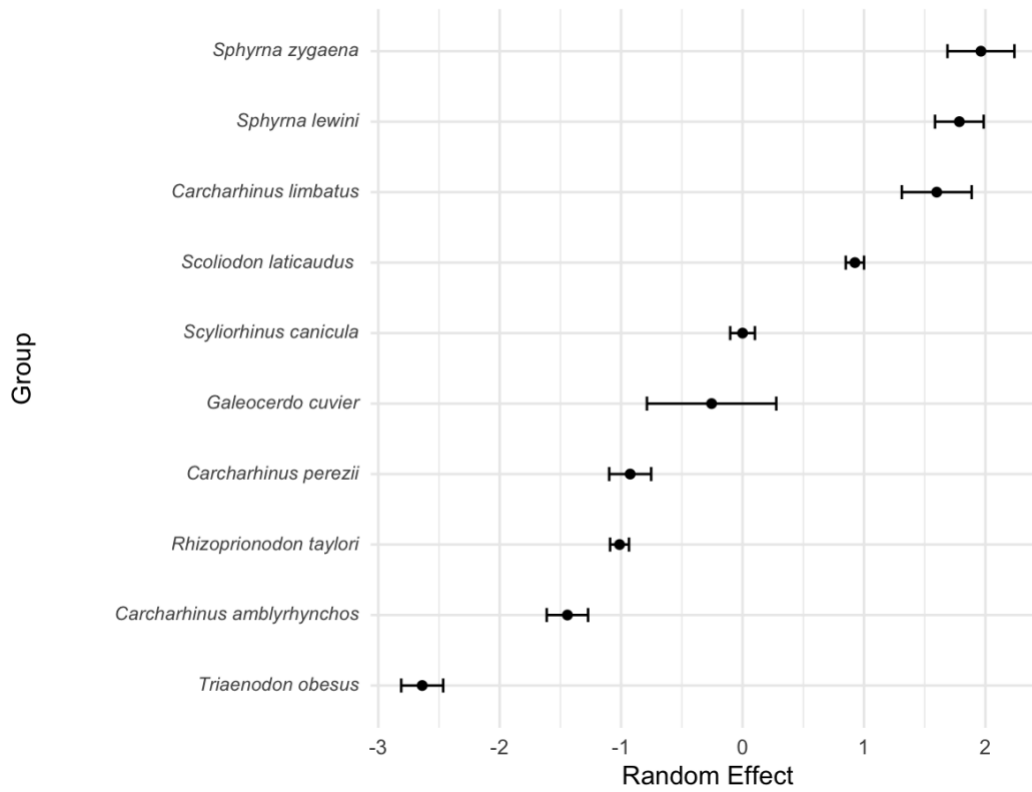


Figure 3.5: Coefficient plots with error bars for a) M9: $\delta^{15}\text{Nc} \sim \text{Total length (cm)} + \text{Population} + \text{Depth (m)} + (1|\text{Species}) + (1|\text{Location})$ and b) M13: $\delta^{15}\text{Nc} \sim \text{Total length (cm)} + \text{Population} + (1|\text{Species}) + (1|\text{Location})$.

a.



b.

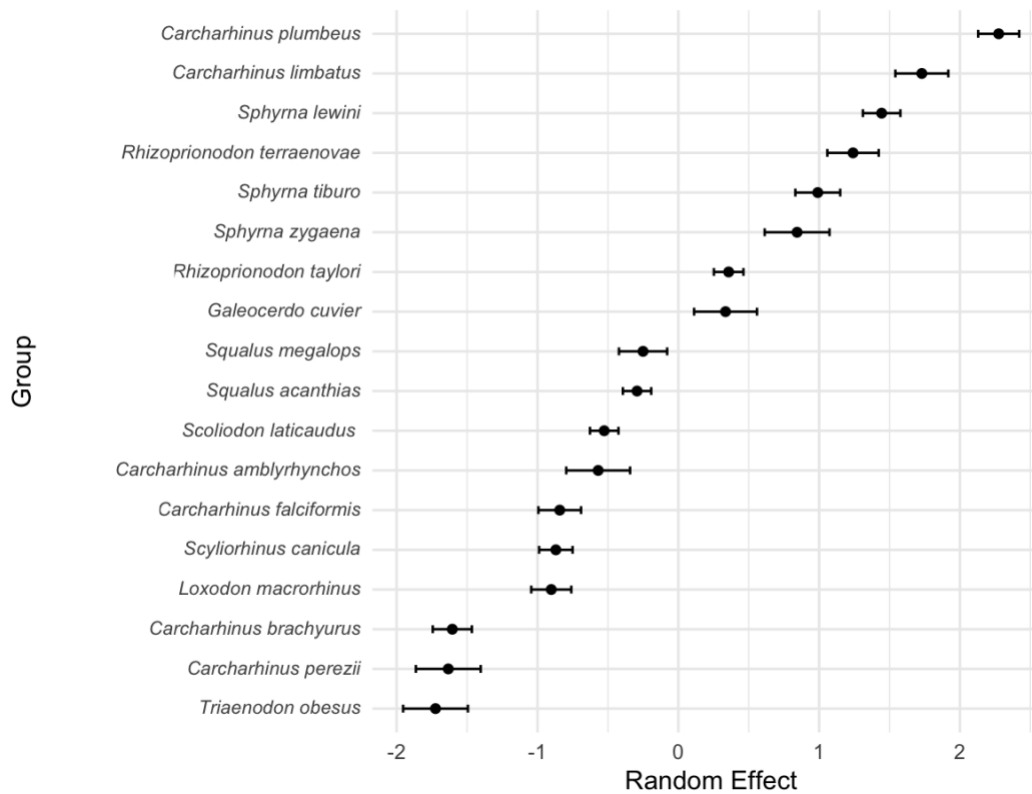


Figure 3.6: Dot plots showing the intercept and error bars of the random effect of species for a) M9: $\delta^{15}\text{Nc} \sim \text{Total length (cm)} + \text{Population} + \text{Depth (m)} + (1|\text{Species}) + (1|\text{Location})$ and b) M13: $\delta^{15}\text{Nc} \sim \text{Total length (cm)} + \text{Population} + (1|\text{Species}) + (1|\text{Location})$.

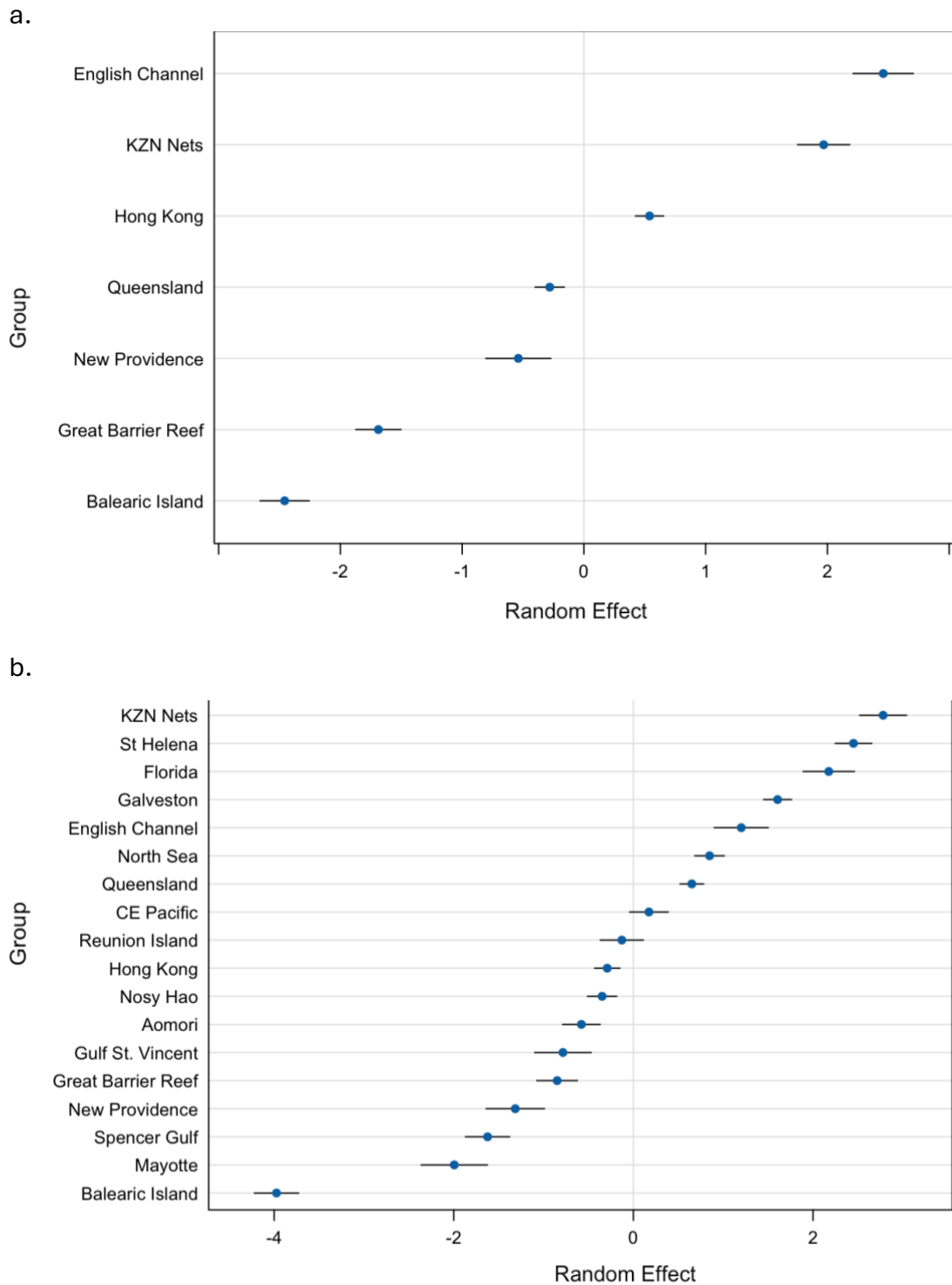


Figure 3.7: Dot plots showing the intercept and error bars of the random effect of location on a) M9 and b) M13.

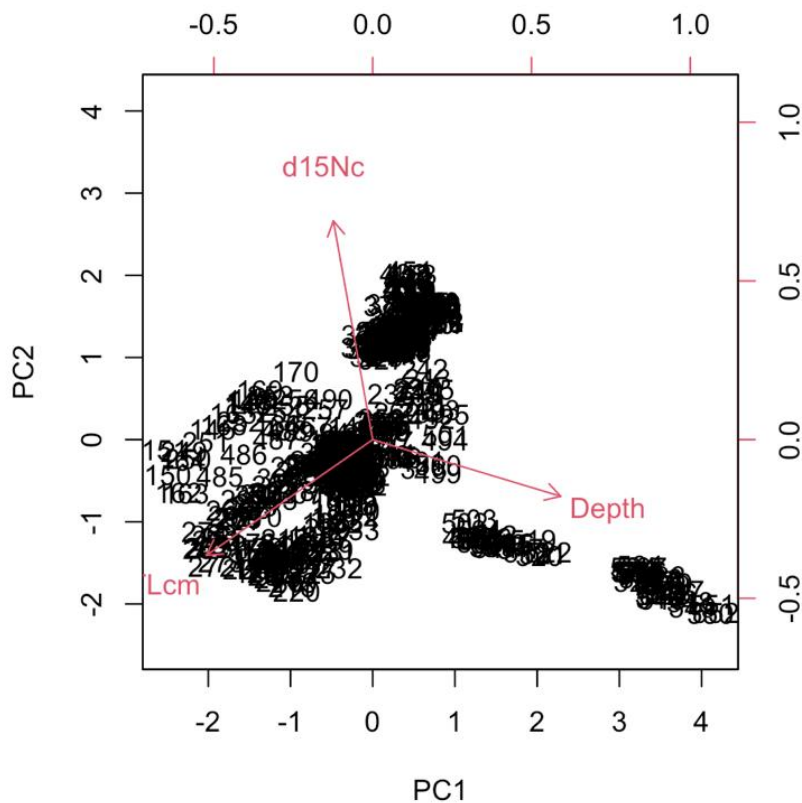
Subsequently, two r^2 values were calculated; marginal (r^2m) and conditional (r^2c). The r^2m value representing the variance explained by the fixed effects in M9 was 0.708, indicating approximately 70.80 % of the variance in $\delta^{15}\text{Nc}$ values is explained by the fixed effects included in this model. The r^2c value representing the variance explained by both fixed and random effects in M9 was $r^2c = 0.963$, indicating approximately 96.30 %

of the variance in $\delta^{15}\text{Nc}$ values is explained by both the fixed and random effects included in this model. For M13 the r^2m and r^2c values were 0.315 and 0.953, respectively.

3.4.3 Principal component analysis

Principal component analysis (PCA) provided a comparison of the variance captured across the fixed effects in M9. In evaluation of the data, principal components (PC) 1 and 2 reported eigenvalues >1 , collectively explaining 81% of the variance (Appendix II, Table 5). Through the PCA vector plot (Figure 3.8a), continuous variables are visually distinguished based on their positions along the principal axes, with corresponding numerical values representing the axis positions for individual samples. Loadings of the continuous variables on PC1 and PC2 were also plotted using PCA and grouped by population category for visual exploration (Figure 3.8b). $\delta^{15}\text{Nc}$ exhibited a strong positive correlation with PC2 (Appendix II, Table 6). Depth demonstrated a strong positive correlation with PC1 and a negative loading on PC2, while TL cm demonstrated a moderate negative correlation with PC1 and PC2 (Appendix II, Table 6).

a.



b.

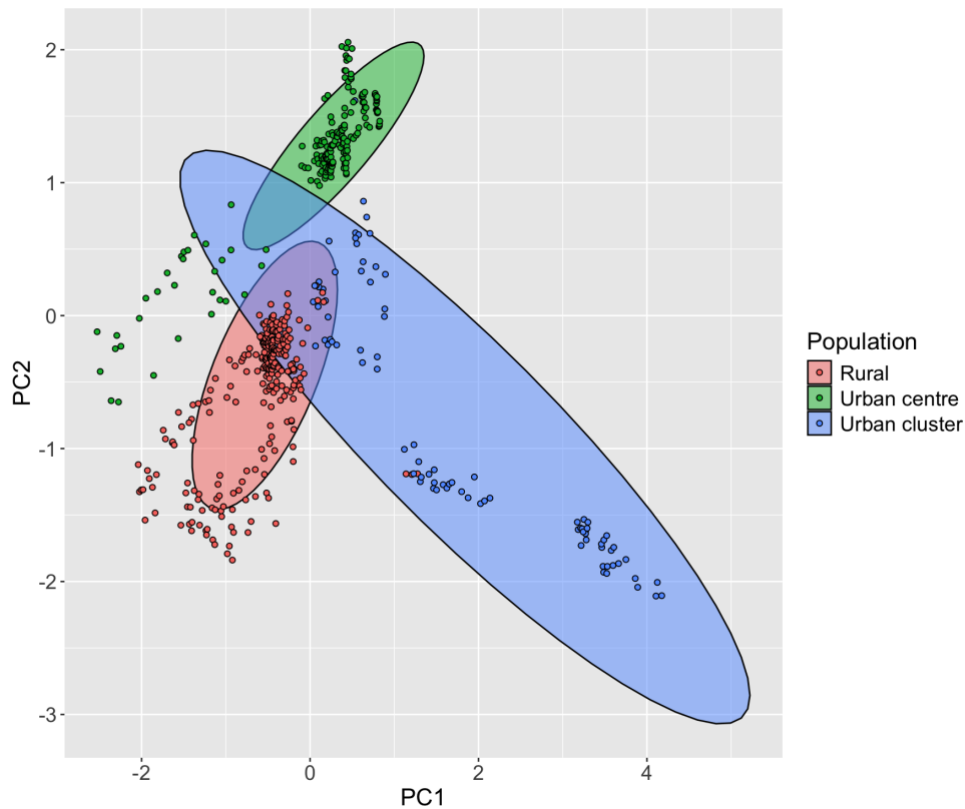


Figure 3.8: Principal component analysis (PCA) visualising a) biplot and b) vector plot performed on the $\delta^{15}\text{Nc}$, depth (m) and total length (TLcm) value (standardised), grouped by population category of survey sites. PC1 explained 43.7% of the total variance and PC2 explained 37.61%.

3.5 Discussion

3.5.1 Fixed effects and $\delta^{15}\text{Nc}$

The preliminary results of this study indicate that $\delta^{15}\text{Nc}$ composition in mesopredatory shark muscle tissue may be significantly influenced by local population characteristics. Notably, sharks sampled near areas characterised as urban centres (Hong Kong and New Providence) exhibited substantially higher $\delta^{15}\text{Nc}$ values compared to those sampled near rural sites. These findings align with previous research suggesting that urbanisation and associated anthropogenic inputs, such as waste water effluents and nutrient enrichment, can elevate $\delta^{15}\text{N}$ levels in marine ecosystems (Cabana and Rasmussen, 1994; Costanzo et al., 2001).

Both the M9 and M13 models estimate that mesopredatory sharks near urban centres had $\delta^{15}\text{Nc}$ values exceeding those of individuals from rural areas by more than 6‰.

Such a difference corresponds to over two apparent trophic levels under the assumption that $\delta^{15}\text{N}$ increases by approximately 2 to 3.4‰ per trophic level in marine food webs (Estrada et al., 2005; Post, 2002). However, caution is needed in interpreting these results, as disparities are also likely to reflect differences in analysis, dietary niches, trophic positioning, nutrient levels, nitrogen assimilation, temporal variations, or altered baseline nitrogen isotope values in urban environments, potentially driven by anthropogenic nutrient sources.

The model incorporating depth data (M9) indicated that urban cluster sites had $\delta^{15}\text{Nc}$ values 2.6‰ higher than individuals sampled near rural sites, while the broader dataset analysed in M13 reported a smaller difference of 1.8‰. Length and depth data exhibited weaker coefficients with $\delta^{15}\text{Nc}$ values compared to rural and urban effects, with length showing a minor positive correlation across both models and depth a slightly negative one in M9. The minor positive effect of total length aligns with studies indicating that $\delta^{15}\text{N}$ values are more strongly influenced by dietary and environmental factors than by body size alone (Kim et al., 2012).

M9 suggests that $\delta^{15}\text{Nc}$ values decrease with depth, however, this finding is largely based on *Scyliorhinus canicula* samples (N = 119, average TL = 42 ± 12 cm collected from depths exceeding 40 m, limiting depth-related conclusions. Notably, *S. canicula* displayed a wide range of $\delta^{15}\text{Nc}$ exceeding 10‰, equivalent to over 3 apparent trophic levels. Samples from the Balearic islands (N = 54, average depth = 118 ± 43 m, TL = 37 ± 7 cm) averaged a $\delta^{15}\text{Nc}$ of 5.4 ± 0.6 ‰, whereas samples from shallower regions in the English Channel (N = 36, average depth = 34 ± 10 m, TL = 41 ± 17 cm) and North Sea (N = 29, depth unknown, TL = 52 ± 6 cm) exhibited higher $\delta^{15}\text{Nc}$ averages of 10.7 ± 1.2 ‰ and 12.2 ± 0.9 ‰, respectively. These differences may reflect variations in habitat, size and diet, but warrant further investigation to clarify their underlying causes.

The more robust AIC value of M9 (AIC = 1297) and its higher r^2 values emphasise the significance of including depth into future isotopic assessments. The fixed effects in M9 explained 71% of the variance in $\delta^{15}\text{Nc}$ values, compared to only 32% in M13 (AIC = 3608), which did not account for depth. This contrast underscores the important role of depth in capturing spatial and ecological variation in nitrogen isotopes.

3.5.2 Random effects and $\delta^{15}\text{Nc}$

Analysis revealed greater variability between species than within species, as well as greater variability between locations than within locations, aligning with expected patterns. The calculated r^2 values, incorporating the random effects of species and location, explains 96% and 95% of the variance in M9 and M13, respectively. This highlights the critical influence of these parameters, although the disparity of the contributions between species and location is notable. Inter-species variance accounted for less than 1‰ in both models, whereas variance between locations ranged between 3-8.6‰ for M9 and M13, respectively. This emphasises the importance of considering a broader range of locations with evenly distributed population densities to better understand if population density directly influences $\delta^{15}\text{N}$ values or if more localised ecosystem processes play a larger role. The wide range of $\delta^{15}\text{N}$ values across species highlights a high degree of trophic plasticity, while the relatively low variation between species indicates a large dietary niche overlap and a level of comparability in $\delta^{15}\text{N}$ values between species occupying similar trophic roles in food webs, consistent with findings in other studies (Estrada et al., 2003; Hussey et al., 2011).

Samples of *T.obesus* (N = 31, average depth = 25 m, TL = 126 ± 19 cm) from the Great Barrier Reef, characterised as a rural location, reported the lowest value for both average $\delta^{15}\text{N}$ ($9.64 \pm 0.77\text{‰}$) and $\delta^{15}\text{Nc}$ ($7.02 \pm 0.77\text{‰}$). In contrast, samples of *R.terraenovae* (N = 50, depth unknown, TL = 89 ± 13 cm) from Galveston, characterised as an urban cluster, reported the highest values for both parameters ($16.04 \pm 0.57\text{‰}$ and $17.70 \pm 0.57\text{‰}$, respectively). Both species share a similar diet of fish, crustaceans and cephalopods (Compagno, 1984), yet habitat difference likely influence nitrogen assimilation and prey availability. *T.obesus* inhabits an oligotrophic reef environments, while *R.terraenovae* is associated with coastal habitats, which may explain some of the isotopic variation (Kim et al., 2012; Papastamatiou et al., 2010), although the $\delta^{15}\text{Nc}$ values should account for habitat-specific nitrogen assimilation to some extent. Additional factors, such as diet-tissue isotope spacing, extraction methods, and analytical methods may also introduce subtle isotopic differences (Hussey et al., 2012). Notably, despite being a relatively small mesopredatory species, *R.terraenovae* exhibits $\delta^{15}\text{Nc}$ values comparable to large apex predators, reflecting a high trophic level (Carlisle et al., 2012; Hussey et al., 2015; Richert et al., 2015).

Significantly high $\delta^{15}\text{Nc}$ values were also observed in *S.tiburo* (N = 50, depth unknown, TL = 98 ± 13 cm) and *C.limbatus* (N = 36, depth unknown, TL = 127 ± 23 cm) species sampled from Galveston, with an average $\delta^{15}\text{Nc}$ of $17.6 \pm 0.56\text{‰}$ and $18.1 \pm 0.53\text{‰}$, respectively. Subsequently, Galveston reported the highest mean $\delta^{15}\text{Nc}$ value of $17.8 \pm 0.59\text{‰}$ across all sites. This was followed by *S.laticaudus* (N = 165, depth = 40 m, TL = 40 ± 14 cm) samples from Hong Kong, with an average $\delta^{15}\text{Nc}$ of $17.6 \pm 0.56\text{‰}$, and *C.perezii* samples (N = 31, average depth = 24 ± 9 m, TL = 146 ± 34 cm) from New Providence with an average $\delta^{15}\text{Nc}$ of $14.8 \pm 0.85\text{‰}$. While all these species share a benthopelagic ecology and primarily feed on fish, crustaceans, and cephalopods, individual feeding strategies and habitat use likely account for some of the observed isotopic variability (Matich et al., 2021). However, considering size-based feeding observations across aquatic predators, *S.laticaudus* appear particularly high (Dalponti et al., 2018; Matich et al., 2021), which could elude to anthropogenic influences from Hong Kong, the most densely populated site in this study (Table 3.3).

The high values of $\delta^{15}\text{Nc}$ reported from Galveston, categorised as an urban cluster, are from 3 species that employ different feeding strategies. *R.terraenovae* and *C.limbatus* primarily feed on teleost fishes (although *R.terraenovae* demonstrate a broader diet including crustaceans and cephalopods), while *S.tiburo* primarily feed on crustaceans (Plumlee and Wells, 2016). This suggests that the high $\delta^{15}\text{Nc}$ observed are more likely to reflect environmental differences than dietary ones. This could be a result of high nutrient inputs from the Mississippi River and other sources along the Gulf of Mexico, which have been linked to increased benthic macroalgae and reduced seagrass density (Seitz and Ewers Lewis, 2018). The northwest Gulf of Mexico is heavily impacted by anthropogenic stressors, including climate change, habitat loss and hypoxia, which have altered biodiversity and ecosystem functioning (Baustian and Rabalais, 2009). These changes likely influence the local marine biochemistry, abundance and distribution of prey species, which would indirectly affect the feeding strategies and isotopic composition of mesopredatory sharks.

3.5.3 Principal component patterns

The PCA analysis also offers insights into how the variables explored relate to the variability in shark muscle data. PC1 and PC2 together explain 81% of the total variance in the data, indicating these two principal components capture most of the patterns observed in the dataset. The PCA clearly delineated distinct groupings corresponding to variations in population categories, which were notably associated with a robust positive correlation between $\delta^{15}\text{Nc}$ and PC2, highlighting the potential importance of distinguishing population categories. The robust positive correlation with depth and PC1 and its negative loading on PC2, also implies depth plays a crucial role in shaping the variance, supporting the model data that $\delta^{15}\text{Nc}$ decreases with depth.

Total length exhibited a moderate negative correlation with both PC1 and a positive correlation with PC2, indicating that larger sharks are inversely associated with depth and body size. Contrary to the slight positive correlation reported in M9, this suggests that larger shark samples are associated with lower $\delta^{15}\text{Nc}$ and shallower depths, where depth data was available. This highlights an intriguing trend: smaller mesopredatory sharks inhabiting shallower depths of regions categorised as an urban centre exhibited higher $\delta^{15}\text{Nc}$ values.

Depth-related differences in $\delta^{15}\text{N}$ values have been observed in other marine taxa, where isotopic gradients reflect changes in food web structure and nutrient cycling across vertical habitats (Hussey et al., 2015; Shipley et al., 2017). The correlations observed may also reflect distinct ecological niches, habitat preferences, or trophic-level differences among populations. However, although an increasing trend in $\delta^{15}\text{N}$ and size might be expected in marine species (Adams and Sterner, 2000; Minagawa and Wada, 1984), dietary niche overlaps in nearshore elasmobranch mesopredators are thought to result from high resource overlap, which may be a key component allowing for high diversity in a system (Vaudo and Heithaus, 2011). Overall, the analyses of the relationship between $\delta^{15}\text{Nc}$ and length across species in this study revealed nuanced trends, underlining the complexities of interpreting ecological dynamics.

Despite these complexities, this study does suggest a discernible trend between $\delta^{15}\text{Nc}$ and coastal regions characterised by high urban population density and provides an

important first look at the isotopic compositions of coastal elasmobranch species through a global lens. Some of these differences may be attributed to various anthropogenic factors, such as wastewater discharges from sewage and agriculture sources (Olsen, 2010). Additionally, topographic, river and hydrological influences, such as nutrient accumulation in bays, are likely to contribute to the observed patterns (Abreu, 2006; Archambault, 1999). This approach could be further developed to provide a cost-effective bioindicator of anthropogenic pollution loading, warranting more detailed exploration.

3.5.4 Model performance and variability

Findings provided insights into the ecological factors influencing $\delta^{15}\text{N}_{\text{c}}$ composition in shark species, emphasising the importance of considering both fixed and random effects in modelling approaches. The model exhibiting the optimal fit encompasses all available parameters, however, while incorporating additional complexity noticeably enhances the model fit it concurrently amplifies uncertainty in contributions. Adding to this complexity, sample sizes vary across parameters, further underscoring the intricacies inherent in this analysis.

The utilisation of baseline nitrogen values from a previous and outdated study also introduces substantial uncertainty into the model. Notably, the presence of low baseline $\delta^{15}\text{N}$ values in regions characterised by high population density amplifies the marked differences in the higher $\delta^{15}\text{N}_{\text{c}}$ values observed across specific locations and species. Moving forward, it is evident that more extensive sampling efforts are warranted, particularly in regions characterised as urban centres. Such efforts should include detailed documentation of capture depth and baseline $\delta^{15}\text{N}$ values, facilitating a more comprehensive understanding of nitrogen dynamics in local environments. Future studies should also consider integrating additional isotopic signatures, such as oxygen and sulphur, and environmental factors, such as proximity to nutrient discharge sites or primary production gradients, to refine the interpretation of $\delta^{15}\text{N}$ variability in mesopredatory sharks.

3.2.5 Sharks as bioindicator species

Indicator species play an important role for understanding the presence and magnitude of pollutants across ecosystems (Parmar et al., 2016). The impacts of marine pollution, including coastal eutrophication and plastic pollution as described in SDG 14, on higher trophic level species have received limited attention (MacLeod et al., 2021). This is partly due to the difficulty in assessing larger organisms *in situ*. Effective bioindicators typically exhibit wide distribution, natural abundance, moderate stress tolerance, and are easily identifiable with feasible sampling methods (Fossi and Panti, 2017; Savoca et al., 2022; Zettler et al., 2013). Incorporating species with diverse habitats and migratory behaviours enhances biomonitoring accuracy and scope (Bonanno and Orlando-Bonaca, 2018; Fossi and Panti, 2017), while identifying high-risk species and populations further refines conservation efforts (Clark et al., 2023).

Sharks are integral to marine ecosystem stability (Libralato et al., 2006). However, nearly a quarter of shark species face threats including overfishing, habitat loss, and declining prey availability (Dulvy et al., 2014; Ferretti et al., 2010). There has been limited exploration of plastic ingestion, entanglement, and bioaccumulation of contaminants concerning elasmobranch species (Afonso and Fidelis, 2023; Bernardini et al., 2018; Cliff et al., 2002), but their significance in pollution assessments is growing (Alves et al., 2022; Bonanno and Orlando-Bonaca, 2018). Sharks' high trophic positions, extensive ranges, and longevity make them effective integrators of biochemical tracers, while their moderate sensitivity to environmental stressors highlights their suitability for monitoring anthropogenic impacts (Gravel et al., 2024).

Sharks' feeding relationships, migrations, and roles in marine food webs are essential for stock assessments and conservation strategies (Ferretti et al., 2010; Rau et al., 1983). SIA has proven effective in evaluating trophic positions and anthropogenic pressures, yet its application in monitoring marine ecosystems remains underexplored (Mancinelli and Vizzini, 2015). Preliminary SIA studies suggest sharks near urbanised areas exhibit altered dietary patterns, reduced diet quality, and elevated $\delta^{15}\text{N}$ values, indicative of nutrient enrichment from anthropogenic sources (Rangel et al., 2021, 2022a).

One of the main objectives of this study was to add to this growing body of knowledge by assessing the potential for pollutant loads to be detectable in the tissues of coastal elasmobranchs and exploring $\delta^{15}\text{N}$ values from an existing database of isotope compositions. While it does not enable the isolation of any specific pollutant or its effects, it offers a global perspective on how anthropogenic pollutants, which are generally associated with high populations, agriculture, and industrialisation, potentially influence shark biochemistry. This initial study warrants further exploration regarding the feasibility of utilising SIA as an indicator for community health. If viable, this approach could be nested within a suite of other monitoring applications and tools to evaluate ecological status (Fossi and Panti, 2017; Maximenko et al., 2021), particularly in resource limited environments. For a comprehensive coastal biomonitoring approach, it would also be pertinent to employ methods capable of capturing signals over both long and short periods of time across both sessile and migratory species, to assess the spatiotemporal extent of anthropogenic impacts.

3.6 Conclusions

This study highlights the potential of mesopredatory sharks as bioindicators for assessing anthropogenic pollution in coastal ecosystems. Despite the fragmented spatial and temporal distributions of the data evaluated, mesopredatory sharks exhibited a strong relationship between $\delta^{15}\text{N}$ values and urbanisation. As apex predator populations decline globally due to overfishing and habitat loss, mesopredatory sharks, many of which are increasing in abundance due to less predatory pressure or targeted are for fisheries, emerge as suitable proxies for evaluating pollution impacts on higher trophic level species.

However, the application of SIA as a tool for environmental monitoring is not without challenges. Marine food webs are highly complex, and interpreting isotopic signatures requires robust baseline data and ecological context. Species-specific variations, historical versus contemporary pollutant exposure, and the influence of dietary and environmental factors necessitate a cautious approach. Advancing SIA methodologies and integrating multiple lines of evidence will enhance the reliability of shark-based bioindicators.

Access to harmonised global nitrogen data, such as through the SDG Indicator 14.1.1a mechanism (UNEP, 2021b), could significantly improve the accuracy of isotopic models and broaden their applicability. Such datasets, encompassing metrics like dissolved inorganic nitrogen (DIN), could serve as baselines for assessing pollution impacts across ecosystems.

Anthropogenic eutrophication effects are particularly pronounced in semi-enclosed systems like bays with high riverine input, where limited circulation exacerbates pollutant accumulation (Wang et al., 2021). These findings underscore the importance of understanding local ecological parameters and species-specific behaviours. Future research should focus on elucidating the effects of urbanisation on isotopic signatures, dietary sources, nitrogen accumulation mechanisms, and ambient nitrogen levels.

Despite challenges, SIA offers a quantitative and ethical method for studying trophic ecology and pollution impacts. Properly calibrated baselines, whether derived from primary consumers, time series of basal resources, or other sources, are critical for effective application. The integration of SIA with existing monitoring could enable the detection of biochemical changes across food webs and the potential to determine threshold levels of impacts, providing a powerful tool for evaluating ecosystem health and addressing global pollution challenges.

In alignment with the United Nation's SDG 14, efforts to reduce marine pollution should consider incorporating innovative approaches like SIA. National-level indicators, such as those for plastic debris, chlorophyll-a, and nutrient concentrations, could complement isotopic studies, facilitating regional and global assessments of pollution hotspots and ecosystem health. By leveraging these tools, we can better understand and mitigate the impacts of anthropogenic pollution on marine ecosystems.

Chapter 4: Rapid assessment of beach litter in the UK: Evaluating transect-based monitoring approaches

4.1 Abstract

This study provides a rapid assessment of anthropogenic litter abundance, distribution, and potential sources across 40 UK beaches, including both mainland and offshore sites. A spatially diverse dataset was generated using two widely applied transect methodologies, covering parallel and perpendicular orientations, to evaluate their effectiveness and comparability. The analysis revealed a mean macrolitter abundance of 0.28 ± 0.46 items/m² for parallel transects and 0.20 ± 0.34 items/m² for perpendicular transects. Mesoplastic abundance was estimated at 0.22 ± 1.28 items/m² and 0.14 ± 0.72 items/m², respectively. Statistical analysis found no significant difference in litter loads between the two methodologies, both with and without mesoplastics ($t_{1,39} = 1.34$, $p = 0.19$). Among litter types, mesoplastics were the most prevalent, followed by fishing and rope related debris, with macroplastics ranking third. While both methodologies produced similar trends in litter abundance and composition, site-specific discrepancies suggest potential bias towards particular litter types. This study highlights the strengths and limitations of each method, emphasising that their suitability depends on the research objectives. Furthermore, it underscores the need for standardised methodologies to enhance the reliability and comparability of marine litter data across monitoring initiatives.

4.2 Introduction

4.2.1 Plastic pollution in the marine environment

Mismanaged plastic waste, particularly plastic packaging, is estimated to account for over 80% of global macroplastic leakage into the marine environment, with littering contributing approximately 5% (Hanke et al., 2019; Lebreton and Andrady, 2019b; OECD, 2023). However, some studies have identified sea-based sources, particularly

those linked to fishing and aquaculture, as predominant contributors to plastic pollution in certain regions (Kaandorp et al., 2023; Krüger et al., 2020; Roman et al., 2020). The considerable spatial and temporal variability in plastic waste generation, sources, and pathways complicates efforts to accurately estimate environmental leakage. Nevertheless, models based on plastic waste generation and assumed leakage rates suggest that between 4 and 23 million metric tonnes (Mt) of plastic waste enter coastal environments annually (Borrelle et al., 2020a; Jambeck et al., 2015a).

Regression models have been widely applied to examine relationships between factors such as population, plastic consumption, waste generation, income levels, local activities, hydrology, and precipitation (Lebreton et al., 2017; Meijer et al., 2021). Some models indicate that macroplastic leakage into the marine environment is highest in rapidly growing economies across Africa and Asia, where waste management infrastructure is often inadequate. However, data from these regions remains scarce (Jambeck et al., 2015a; Lebreton and Andrady, 2019b).

Additionally, plastic consumption is significantly higher in high-income countries, which frequently export plastic waste to regions with inadequate infrastructure and weaker regulatory enforcement (Gündoğdu and Walker, 2021). This practice exacerbates global disparities in the responsibility for mismanaged waste. Despite a severe lack of waste infrastructure, plastic production is expected to increase from >400 to >800 million Mt by 2050, which could triple the amount of globally mismanaged plastic waste (Dokl et al., 2024; Lebreton and Andrady, 2019a).

Rivers have been recognised as significant pathway for marine plastic pollution, with small urban rivers playing a more substantial role than previously recognised (Al-Zawaidah et al., 2021; Meijer et al., 2021). However, riverine data is limited, with temporally restricted measurements available for only approximately 100 rivers out of more than a million worldwide. Subsequently, models estimating plastic emissions and mass balance can vary by two to three orders of magnitude (Kaandorp et al., 2023; Zhang et al., 2023). Despite these uncertainties, the spatial patterns of plastic pollution in the marine environment suggest that a substantial portion accumulates throughout the water column, while the seafloor retains non-buoyant and slow degrading materials (Harris et al., 2023; Martin et al., 2022; Roman et al., 2020). The diverse transport routes

of plastic pollutants across terrestrial and aquatic ecosystems, driven by material properties, currents, waves, and wind, further highlight the challenges of effective monitoring (Eriksen et al., 2014b).

4.2.2 Beach litter monitoring

Beach litter surveys are among the most widely used method for monitoring plastic pollution due to their accessibility and effectiveness (Haarr et al., 2022). Although beaches account for less than 0.02% of the global marine environment, they may accumulate up to 2% of global marine plastics (Kaandorp et al., 2023; Rieger et al., 2024). As natural sinks for marine debris, beaches provide easily accessible locations for assessing the types, quantities, and sources of plastic pollution entering marine environment, offering valuable insights for research and policy efforts (Addamo et al., 2017; Binetti et al., 2020; Hanke et al., 2019). Additionally, beach litter monitoring is a cost-effective approach that can be supported by trained citizen scientists, who not only contribute to data collection but also help raise public awareness and expand datasets through community participation.

The United Nation's Sustainable Development Goals (UN SDGs) recommend national monitoring of beach litter under indicator 14.1.1b, which includes floating plastics, water column plastics and seafloor litter. Supplementary indicators, including plastic ingestion by biota, entanglement, riverine litter, and microplastic pollution, are also encouraged (UNEP, 2021b). Monitoring guidance for SDG indicator 14.1.1b refers to the Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) guidelines, which outline widely used monitoring methodologies for monitoring marine litter across different environmental compartments (GESAMP, 2019; UNEP, 2021b). These guidelines assist surveyors with selecting appropriate methods based on specific research or policy objectives.

Despite these frameworks, studies and initiatives often adopt tailored methodologies to accommodate project-specific needs and resource availability, making it difficult to standardise marine litter monitoring. As a result, evaluating long-term marine litter trends remains challenging, as survey methods range from clean-up-based approach to systematically designed quantitative assessments. The latter prioritise data reliability

over litter removal and commonly employ quadrats, transects, or area-based surveys (COBSEA and CSIRO, 2022).

Beach litter surveys are generally classified into two main types: accumulation surveys and standing stock surveys (GESAMP, 2019). Accumulation surveys measure the rate of litter deposition over time by periodically clearing and re-measuring a defined area, providing insights into litter flux and temporal trends. However, these surveys require repeated site visits and are influenced by local conditions, making them labour-intensive and site-specific. In contrast, standing stock surveys provide a snapshot of the total litter present at a given moment, capturing both recent and long-standing debris. While these surveys are less resource-intensive and facilitate site comparisons, they do not account for temporal variability and may overestimate litter input rates if legacy pollution is significant (Rieger et al., 2024).

Additionally, two distinct transect-based survey approaches are widely applied in key regional and national frameworks for quantifying beach litter. In this study, these are referred to as the parallel and perpendicular transect survey approaches.

4.2.3 Transect approaches

Transect surveys involve using a transect tape to systematically record observations across a designated area. Surveyors walk along a straight line, known as the transect line, and count and/or weigh items of interest within a fixed distance from the line. Although this method is relatively straightforward, challenges arise in accurately estimating the total count and weight of litter, as well as in defining the upper limit of the shoreline (GESAMP, 2019). Ensuring that data collected across survey sites are both representable and comparable is a common challenge for all monitoring methods. However, when sites and transects are properly selected, transect surveys can provide an efficient, cost effective, and robust means of sampling diverse locations (COBSEA and CSIRO, 2022; Lavelle et al., 2024).

There are two primary transect approaches for monitoring beach litter. One of the most widely used methods across Europe involves a 100m transect parallel to the water's edge, which typically reports data as items and/or dry weight of items per 100m.

However, if the total transect area is known, this can be easily transformed into items or dry weight per m². This approach is endorsed by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) and the European Union's Marine Strategy Framework Directive (EU MSFD) (2008/56/EC) (European Commission, 2023; Wenneker and Oosterbaan, 2010). It has also been applied to a number of studies across Africa (Lavelle et al., 2024; Okuku et al., 2020; Ryan, 2020) and the Pacific (Binetti et al., 2020).

Conversely, in North America, the Caribbean, East Asia, and Oceania, perpendicular transects extending from the water's edge to the back of the beach are more commonly used (Burgess et al., 2021; CSIRO, 2021; CSIRO et al., 2021, 2021; CSIRO and Earthwatch Australia, 2021). This approach reports data as items and/or dry weight per m² and cannot be easily transformed to align with the EU's proposed indicator of <20 beach litter items per 100m (Hanke et al., 2019).

In addition to quantification differences, harmonisation across these two approaches is complicated by inconsistencies in defining litter size classes and transect areas (COBSEA and CSIRO, 2022; GESAMP, 2019). Perpendicular transect guidance typically suggests a fixed transect width (often 1m, 2m or 5m), whereas parallel transects are generally conducted across the entire beach compartment, from the water's edge to the back of the beach (COBSEA and CSIRO, 2022; Wenneker and Oosterbaan, 2010).

Despite these recommendations, variations persist in survey design. Some studies collect data from specific sections, such as below and/or above the upper strandline, while others extend 2 m into the vegetation or the upper shoreline (Brennan et al., 2018; Velandier and Mocogni, 1999). Additionally, while guidelines recommend conducting monthly or seasonal surveys to obtain representative data, monitoring efforts often remain fragmented spatially and temporally, largely due to resource constraints.

Some transects sample areas where litter naturally accumulates, while others include relatively bare sections, leading to potential inconsistencies (Galgani et al., 2015). Litter accumulation on beaches is also heavily influenced by tidal cycles, storm events, and seasonal variations, which further complicated efforts to assess long-term trends. To ensure meaningful comparisons, data collected from strandlines should primarily be

used to analyse similar environments, such as beaches with comparable morphology, orientation, and proximity to litter sources. However, the perpendicular transect approach is particularly valuable for comparing different environments, including riverbanks and roads (COBSEA and CSIRO, 2022; GESAMP, 2019).

A critical challenge arises from inconsistencies in regional and global reporting mechanisms. For example, SDG 14.1.1b requests marine litter data to be reported in items km² (UNEP, 2021b), yet extrapolating localised beach surveys to represent broader spatial scales can introduce misleading conclusions. Without greater alignment between study methodologies and monitoring frameworks, regional and global marine litter assessments remain inconsistent, ultimately hindering efforts to mitigate plastic pollution effectively.

4.2.4 Research questions

This study employed both perpendicular and parallel transect survey approaches to rapidly assess the abundance (standing stock) and distribution of beach litter across the UK. The primary aim was to analyse litter data from a variety of UK beaches and quantify the differences between these two widely used methodologies, both of which are recommended by GESAMP (2019).

The research sought to address the following key questions:

- What are the current volumes, distributions and compositions of beach litter across UK beaches, based on a post-COVID summer snapshot survey?
- Are there spatial patterns or similarities in the distribution of marine litter categories across surveyed sites?
- Do different transect approaches yield significantly different results?
- Are certain survey methods biased towards detecting specific types of litter?
- How does recorded litter data compare to existing datasets and observed trends for marine plastic pollution?
- What are the primary sources of beach litter in the UK, and what management considerations arise from these findings.

4.3 Methodology

4.3.1 The vessel of opportunity

The ‘Darwin 200’ project was an initiative supported by single use plastic (SUP) campaigners City to Sea, Sea Sanctuaries Trust and Seas Your Future. Seas Your Future are a charitable organisation that funds young people to undertake sail and environmental related activities to develop their physical, mental and social capabilities on a tall ship, the Pelican of London. The Pelican subsequently provided a vessel of opportunity to conduct a marine litter survey, supported by volunteers, including graduate and undergraduate marine scientists. Following two days sail training, the Pelican disembarked on the 17th of May 2021 from Folkestone on a 13-week expedition circumnavigating the UK.

4.3.2 Training

The monitoring approach developed for this study utilised graduate and undergraduate researchers as survey leads, as well as citizen scientists who were paired with survey leads for supporting data collection. Training covered basic research background of marine litter, the scientific approach to the study, data collection, data entry, and safety requirements (particularly regarding COVID-19). Following training, informal tests were conducted to ensure volunteers’ confidence in their understanding and to maintain data quality.

4.3.3 Study area

The UK has one of the longest coastlines in Europe, with the mainland stretching over 17, 000 km, which almost doubles when including the thousands of islands (EUROSION, 2004). The expedition witnessed a diverse range of the UK’s coastal formations, including sandy beaches, mud flats, sand dunes, rocky outcrops and fjord coastlines. This diversity is shaped by millennia of geological processes, such as rock erosion and multiple glaciations (May and Hansom, 2003). Today, many parts of the coastline also owe its formation to human-related activities such as resource-use,

harbour construction and coastal protection. Notably, 45 % of England's coastline has been developed with coastal defences and artificial beaches (Masselink et al., 2020).

The UK has a humid temperate oceanic climate, characterised by four seasons and pronounced regional variations (Peel et al., 2007). Western coasts endure harsher winters, frequent storms, and higher precipitation due to Atlantic influences, while eastern coasts are more sheltered, affected by cold, dry air masses from continental polar regions (Hanna et al., 2017). The northwestern coasts, including Scotland, Orkney and Shetland, are the windiest areas due to polar air masses, while the Isles of Scilly off the southwestern coast demonstrate a mild oceanic climate bordering on humid subtropical, supporting unique biodiversity (Parslow, 2007). The south coast also experiences significant winter storms driven by Atlantic weather systems and jet stream shifts (Hanna et al., 2017).

The expedition started during unseasonably extreme weather, with several force 9 storms confining the vessel to sheltered bays along England's south coast. May 2021 saw the second-highest rainfall on record, with the southwest and northeast receiving double the monthly average, following an unusually dry April (Met Office, 2021). Despite recent trends toward warmer and wetter conditions, the UK climate remains marked by notable seasonal variability (Kendon et al., 2022).

4.3.4 Site selection

The vessel circumnavigated the UK starting at Folkstone on the Southeast coast and finishing in London, travelling over 5,000 km. The study area included a total of 40 beaches surveyed around the coast of the UK between May-August 2021 (Figure 4.1). Site selection was primarily dictated by the vessel's predetermined route and prevailing weather conditions, resulting in the selection of predominantly sheltered survey sites.

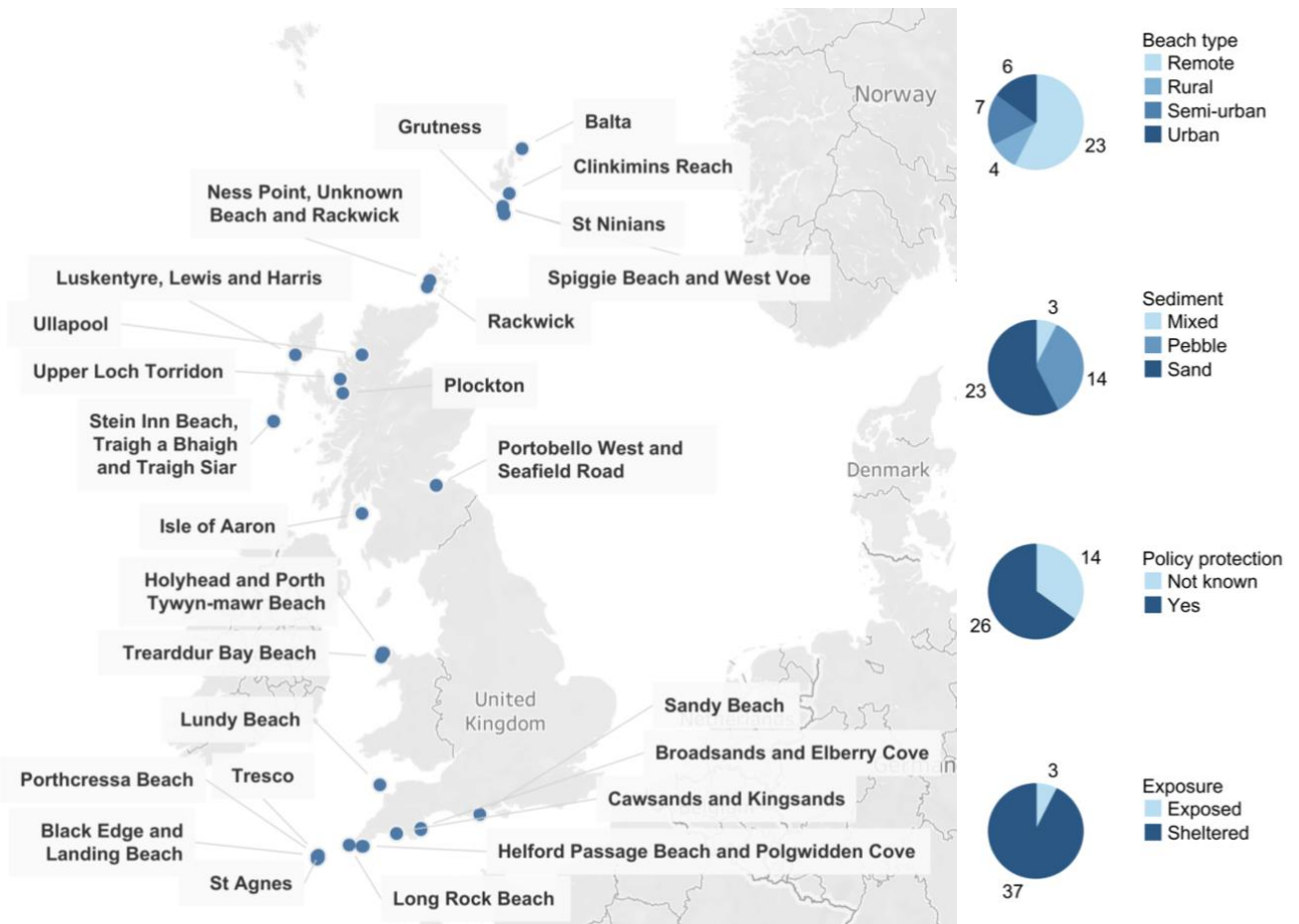


Figure 4.1: Beach litter survey sites ($N = 40$) and characteristics from the Pelican of London's route around the UK, between May and August 2021.

A site description form documented local activities and beach morphology to help assess potential sources and variations in marine litter. Beaches were categorised by sediment type, exposure, and if a local level of environmental protection was present (Figure 4.1). Most sites were sand (53%), sheltered (93%), and located within areas under some level of policy protection (65%).

In the UK, Marine Protected Areas (MPAs) are designated into Marine Conservation Zones (MCZs), Nature Conservation Marine Protected Areas (Scotland only), Special Area of Conservation (SACs) and Specially Protected Areas (SPAs), each with different objectives and policies (Anbleyth-Evans and Williams, 2018).

Additionally, most sites were remote (53%), based on the Bathing Area Registration and Evaluation (BARE) approach (Micallef and Williams, 2004; Rangel-Buitrago, 2018; Vaz, 2009) (Figure 4.1, Table 4.1).

Table 4.1: Beach categorisations as defined by the Bathing Area Registration and Evaluation (BARE) approach (Vaz, 2009)

Beach type	
Urban	Next to major marinas, fishing ports, constructions, high number of users during season, wide range of beach equipment
Semi-urban	Surrounding urban area, large number of users in season, wide range of beach equipment, car parking
Rural	Near small town/village, natural features outnumber manmade elements, some access, limited beach equipment
Remote	Natural environment with scarcely any trace of human intervention

Most sites (68%) were located on offshore UK islands across the Isles of Scilly, Lundy, Anglesey, the Hebrides, Orkney and Shetland Isles (Figure 4.1). Additionally, most sites (75%) were found to be subject to some level of clean-up operation, with two instances where clean-ups were going on during the surveys (Sandy Beach and Long Rock Beach). Clean-up efforts were understood to be conducted by volunteers and local authorities, with varying frequencies. Details of site descriptions are provided in the Appendix III (Table 1).

4.3.5 Litter quantification and categorisation

This study drew upon methodological guidelines from OSPAR (2010), CSIRO (2020), and NOAA (2021), employing both parallel and perpendicular transect approaches. Surveys were conducted at least 50m from access points to minimise bias.

Before collecting litter data, GPS coordinates were recorded to establish reference points for the start and end of each transect, with a positional accuracy of $\pm 10\text{m}$. Monitoring ideally occurred approximately one hour after high tide, although logistical

constraints occasionally impeded this timing. Each survey was conducted by no more than two individuals to ensure consistency and accuracy.

For parallel transects, a 100m stretch parallel to the water's edge along the high tide line (strandline) defined the sampling unit, while perpendicular transects comprised 3-6 transects extending from the water's edge to 2m into the vegetation at the back of the beach, unless obstructed (Figure 4.2). Sampling units were a minimum of 50m apart.

For parallel transects, litter within 1m on either side of the strandline was documented, while for perpendicular transects, litter within 1m either side of the transect tape was recorded (Figure 4.2). In cases of varying sediment types at a site, transects were allocated proportionally among them.

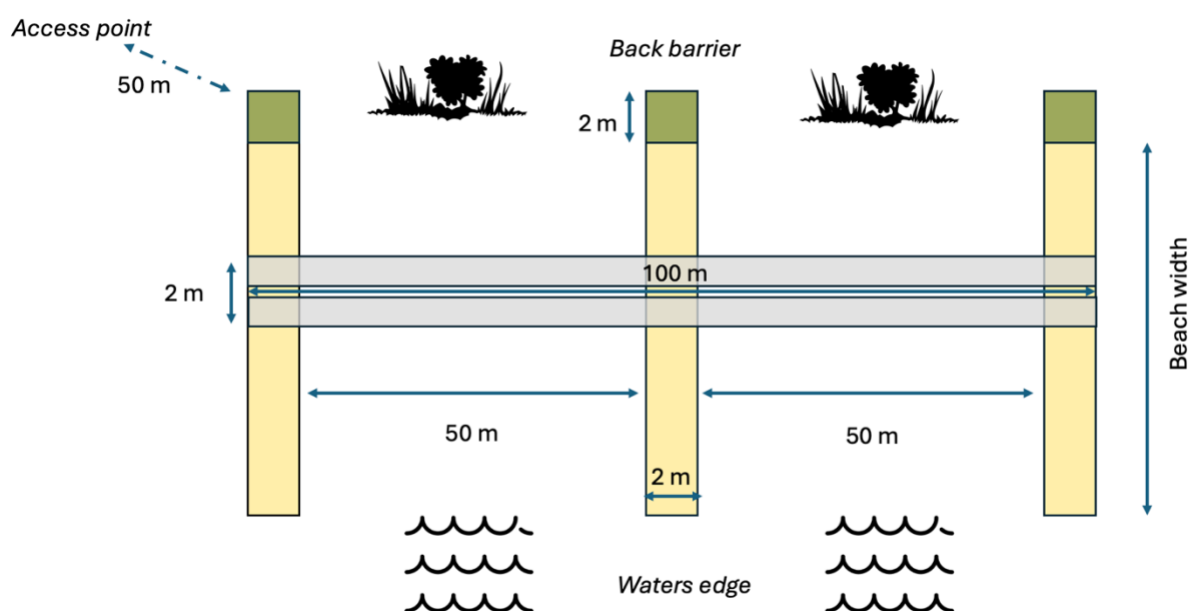


Figure 4.2: Diagram illustrating the parallel (grey) and perpendicular (yellow) transect approaches for beach litter surveys.

Within these sampling units, all macrolitter (>2.5cm) and mesolitter (5mm to <2.5cm) was counted. Litter categorisation adhered to the template outlined by OSPAR (2010). All litter was identified and recorded by specific item type and material (encompassing plastic, paper and cardboard, wood, rubber, textile, metal, ceramic, and other materials). Data was quantified as items/m² for comparison.

It should be noted that considering the 100m approach, due to logistical constraints, data collected in this study sub-sampled a 2m wide section around the strandline. Therefore, it is not directly comparable to methodologies surveying the entire beach width and/or tidal compartments.

4.3.6 Data analysis

All data underwent cleaning and quality assurance checks before analysis. Statistical analyses were conducted using R and Tableau Public, both of which are free statistical analysis and data visualisation tools.

As recorded data was not normally distributed, parametric, correlation, and multivariate analyses were performed on log-transformed data ($\log x + c$) to analyse abundance data reported between transect approaches. An *F*-test was applied to evaluate variance, followed by a *t*-test to compare mean litter abundance reported across each method. Linear regression was used to assess correlations in abundance data across material categories, while Procrustes analysis was applied to quantify the alignment of material abundance reported between approaches by assessing their similarity. A low m^2 value indicates that the two methods report similar patterns.

Hierarchical clustering was performed using Ward's grouping method with Euclidean distance to assess similarities in relative litter abundance across survey sites for both transect approaches. Additionally, PERMANOVA (Permutational Multivariate Analysis of Variance) was applied to evaluate significant differences in material abundance and composition, while SIMPER (Similarity Percentage Analysis) identified the contribution of each material category to the total difference between methods. It is important to note, SIMPER percentages represent relative contributions to overall dissimilarity rather than absolute amounts of each material.

Principal Coordinate Analysis (PCoA) was also conducted on a Bray-Curtis distance matrix to explore the relative composition of material categories across sites for both survey methods. PCoA ordination visually represents site relationships, with distances reflecting dissimilarities in relative material composition. If material categories were significantly correlated ($p = \leq 0.05$) with the ordination axes, vector arrows were

projected onto the PCoA plot, indicating the direction and magnitude of their influence on clustering patterns. A Hellinger transformation was applied to normalise relative abundance data for PCoA.

For each value x_{ij} (where i is the site and j is the material category), the transformation is defined as:

$$x'_{ij} = \frac{\sqrt{x_{ij}}}{\sqrt{\sum_j x_{ij}}}$$

Where:

- x_{ij} is the original abundance of material j at site i ,
- x'_{ij} is the transformed value,
- $\sum_j x_{ij}$ is the total abundance of all materials at site i .

Redundancy analysis (RDA) was performed on log-transformed data to investigate relationships between the average abundance of commonly littered materials and site variables (location, beach type, clean-up effort, and policy protection). RDA, a canonical form of Principal Component Analysis (PCA), is a direct gradient analysis method. Similar to a PCA biplot, RDA allows for interpreting the patterns of litter abundance across sites and the direction of variation associated with site variables.

Values are reported as means \pm standard deviation (SD), unless stated otherwise.

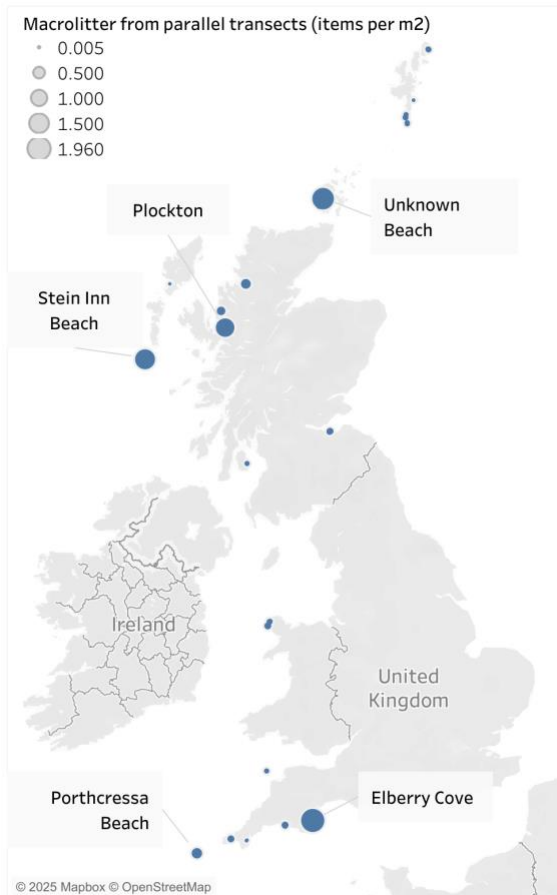
4.4 Results

4.4.1 Total litter abundance reported across both transect approaches

Litter was recorded at all survey sites (Figure 4.3). In total, parallel surveys recorded 2,352 macrolitter items and 1,793 mesoplastic items, while perpendicular surveys documented 1,995 macrolitter items and 821 mesoplastic items. Notably, the Plockton site (northwest coast of Scotland) accounted for the majority of mesoplastic litter (<2.5

cm), contributing 90% and 77% of the total mesoplastic counts in parallel and perpendicular transects, respectively. The dominant mesoplastic items at this site were polystyrene fragments, likely originating from localised packaging leakage, along with a small number of plastic production pellets.

a.



b.

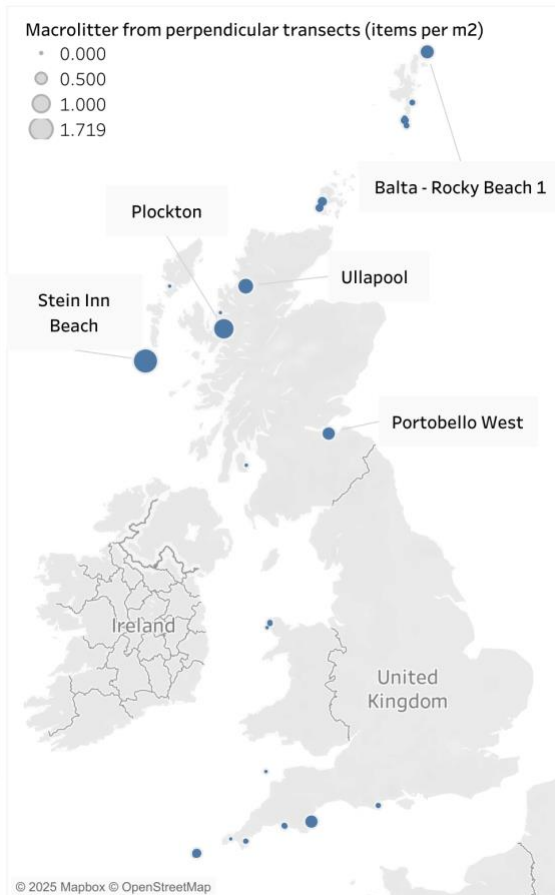


Figure 4.3: Maps illustrating total macrolitter abundance (items m²) recorded across beach survey sites (N = 40) from a) parallel transect surveys and b) perpendicular transect surveys. The five sites with the highest reported abundances are labelled.

The total surveyed area differed between methods, with parallel transects covering 9,100 m² and perpendicular transects covering 11,436 m². The mean macrolitter abundance across all sites was estimated at 0.28 ± 0.46 items/m² for parallel transects and 0.20 ± 0.34 items/m² for perpendicular transects. The mean mesoplastic abundance was estimated at 0.22 ± 1.28 items/m² across parallel transects and 0.14 ± 0.72 items/m² across perpendicular transects.

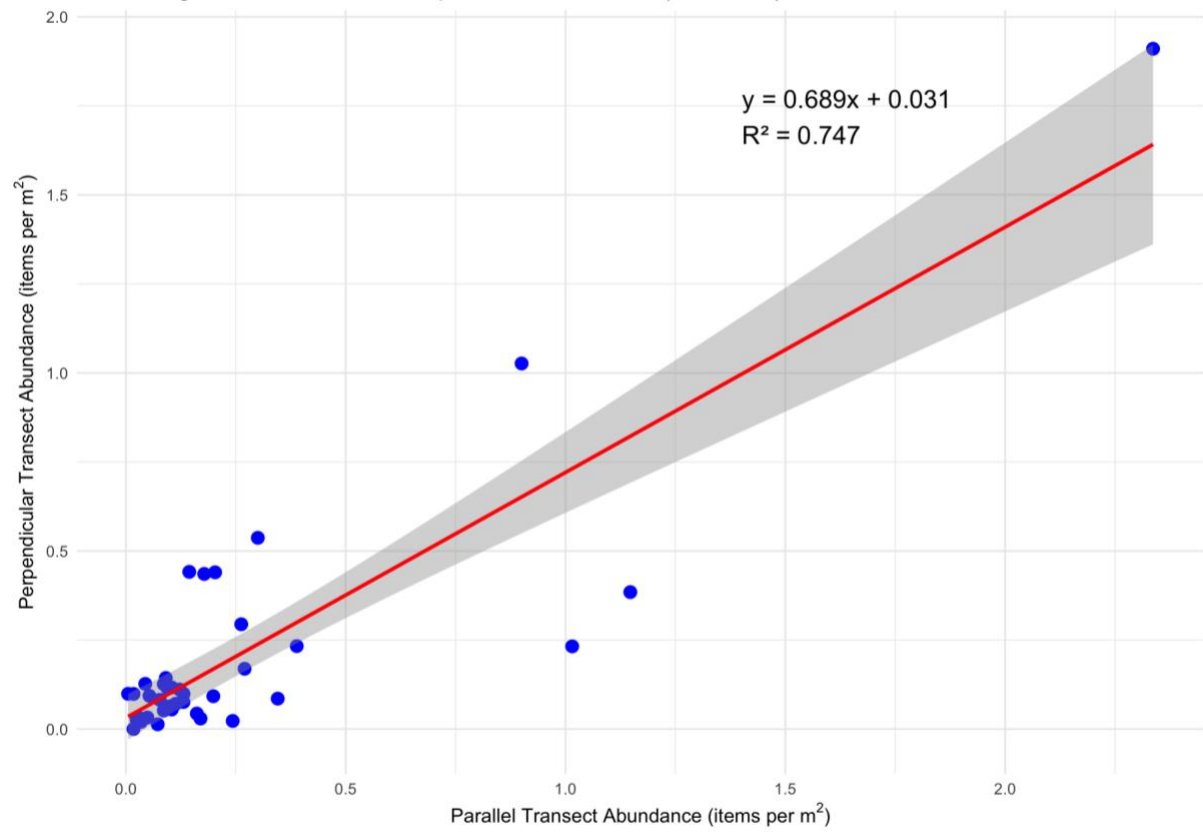
Variability in total macrolitter litter estimates was higher in parallel transects, as indicated by a higher total sum of squares value ($SS_{Total} = 8.43$) and mean square value ($MS = 0.22$) compared to perpendicular transects ($SS_{Total} = 4.39$ and $MS = 0.11$, respectively).

An F -test reported no significant difference in variance between the two transect approaches, both when including mesoplastics ($F = 1.57$, $p = 0.92$) and when excluding them ($F = 1.63$, $p = 0.93$). A subsequent t -test reported no significant difference in mean litter abundance between transect approaches across sites, both when including mesoplastics ($t_{1,39} = 1.34$, $p = 0.19$) and excluding them ($t_{1,39} = 1.34$, $p = 0.19$).

At a site level, linear regression identified a significant relationship between parallel and perpendicular transect counts when including mesoplastics ($F_{1,38} = 112.3$, $p < 0.01$), with a strong correlation ($r^2 = 0.75$) and a parallel coefficient of 0.68 ($p < 0.01$) (Figure 4.4a). The model remained significant when excluding mesoplastics ($F_{1,38} = 37.27$, $p < 0.01$), but with a weaker correlation ($r^2 = 0.50$) and a reduced parallel coefficient (0.55, $p < 0.01$) (Figure 4.4b). The intercept was not significant in either model, suggesting no systematic offset when litter abundance is zero.

Procrustes analysis further indicated a moderate degree of similarity between transect approaches when excluding mesoplastics ($m^2 = 0.50$, $r^2 = 0.70$, $p = 0.001$). When including mesoplastics, the similarity increased ($m^2 = 0.25$, $r^2 = 0.86$, $p = 0.001$). Detailed litter abundances of each site for each transect approach are provided in Appendix III (Table 1).

a.



b.

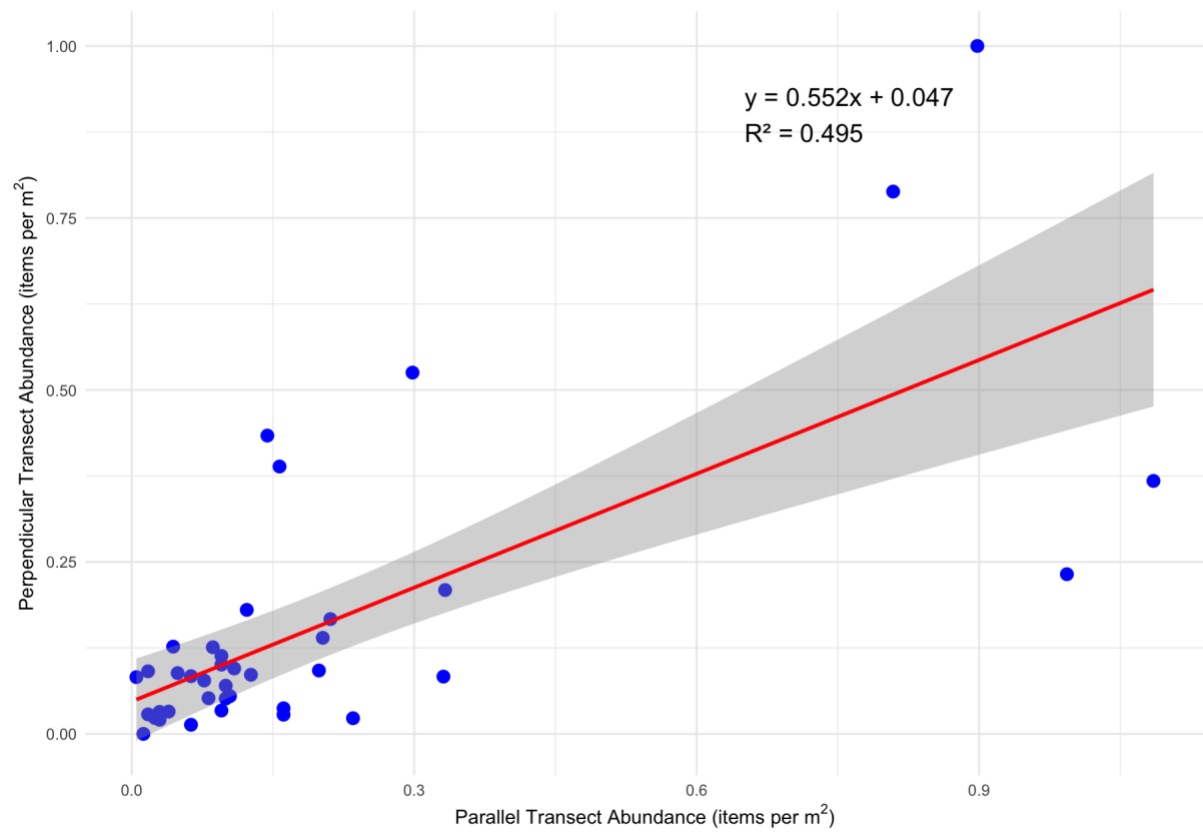
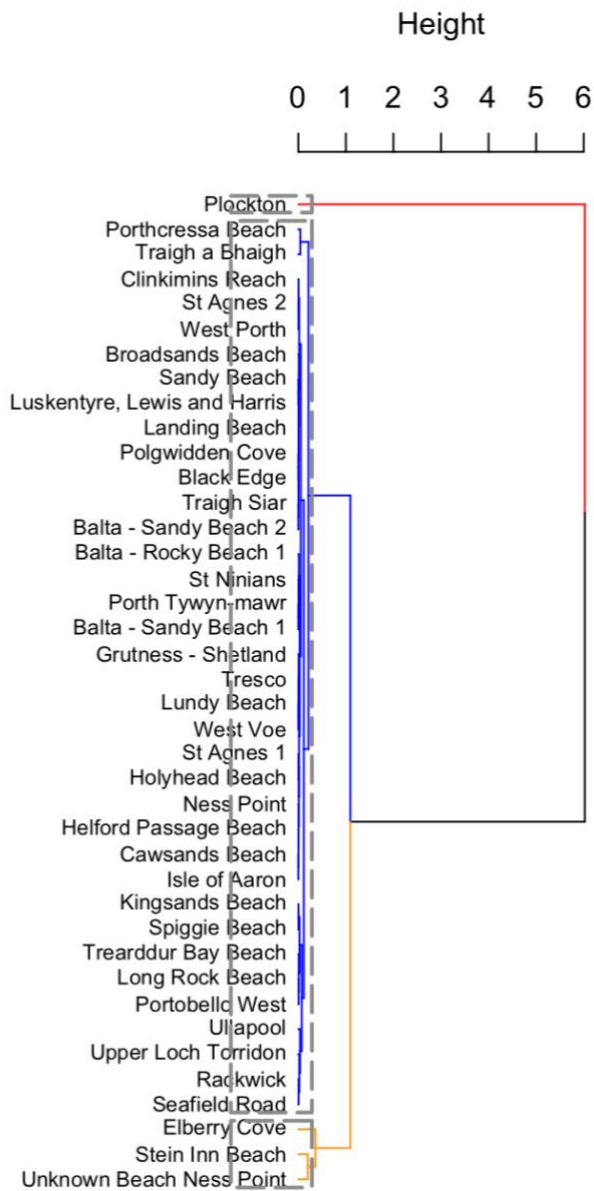


Figure 4.4: Linear regression showing log transformed abundance (items per m²) of beach litter a) including mesolitter and b) excluding mesolitter reported from parallel and perpendicular transect surveys across beach sites (N = 40), reporting a Pearson's correlation of 0.75.

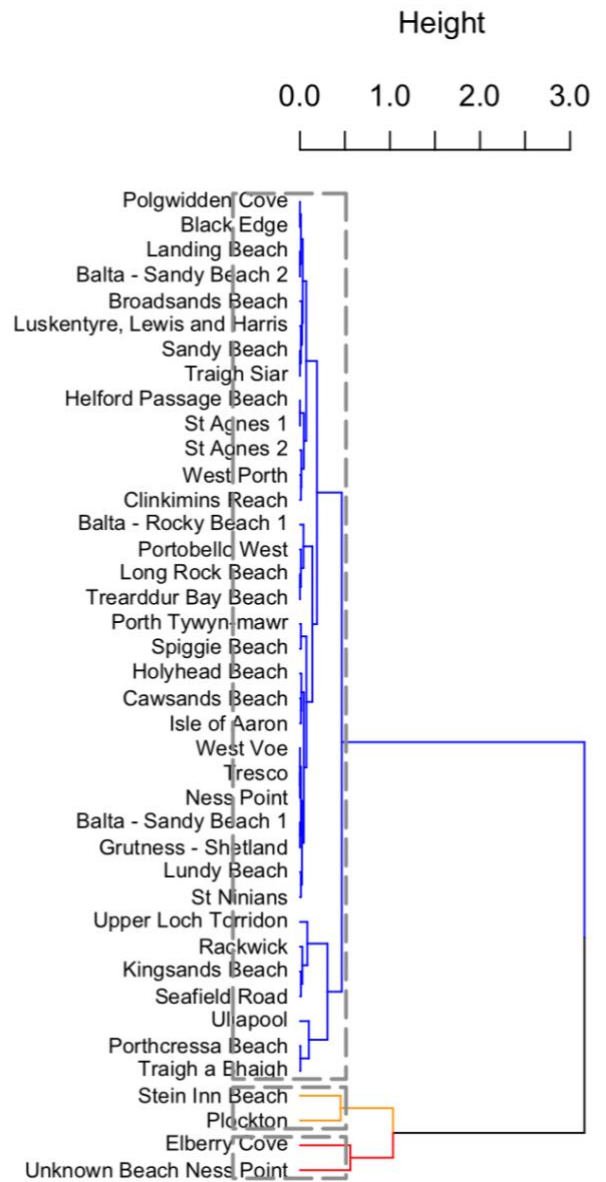
Hierarchical clustering analysis (AHC) revealed similar major clusters across both transect approaches when considering beach sites and total litter recorded (Figure 4.5). Both transect approaches identified Plockton as the most polluted site when including mesoplastics (Figure 4.5a and Figure 4.5c). When excluding mesoplastics, parallel transects identified Elberry Cove and an unknown beach at Balta as the most polluted sites based on macrolitter abundance, with a secondary cluster grouping Plockton and Stein Inn Beach (Figure 4.5b). These four locations accounted for 54% of the total macrolitter (71% including mesoplastics) recorded in parallel transect surveys.

In the perpendicular transects, Stein Inn and Plockton are identified as the most polluted sites based on macrolitter abundance (Figure 4.5d), contributing 33% of the total macrolitter (85% including mesoplastics).

a.



b.



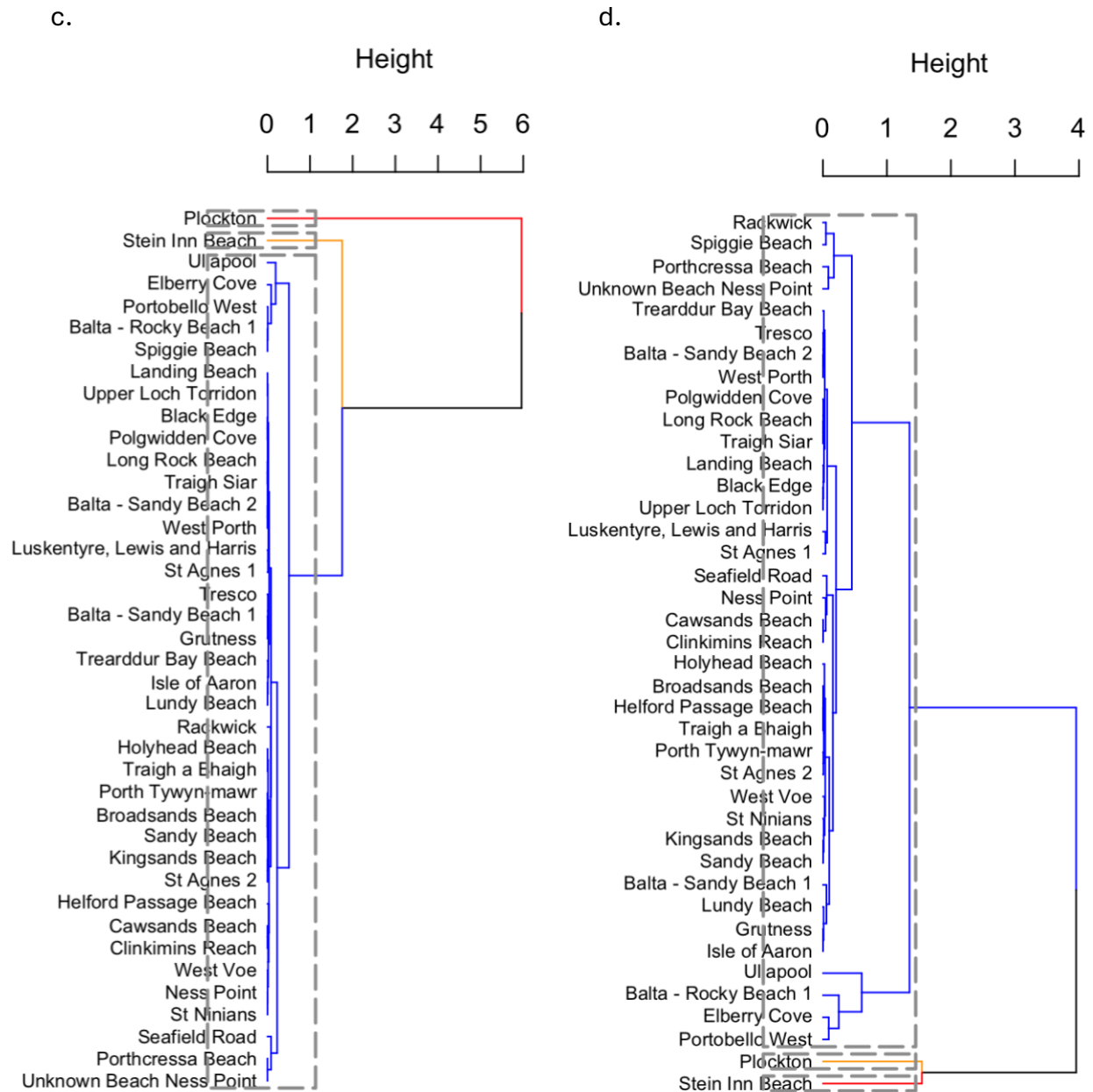


Figure 4.5: Agglomerative Hierarchical Clustering (AHC) to illustrate litter abundance data (items per m^2) across beaches surveyed ($N = 40$) for a) parallel transects including mesoplastics, b) parallel transects excluding mesoplastics, c) perpendicular transects including mesoplastics, and d) perpendicular transects excluding mesoplastics.

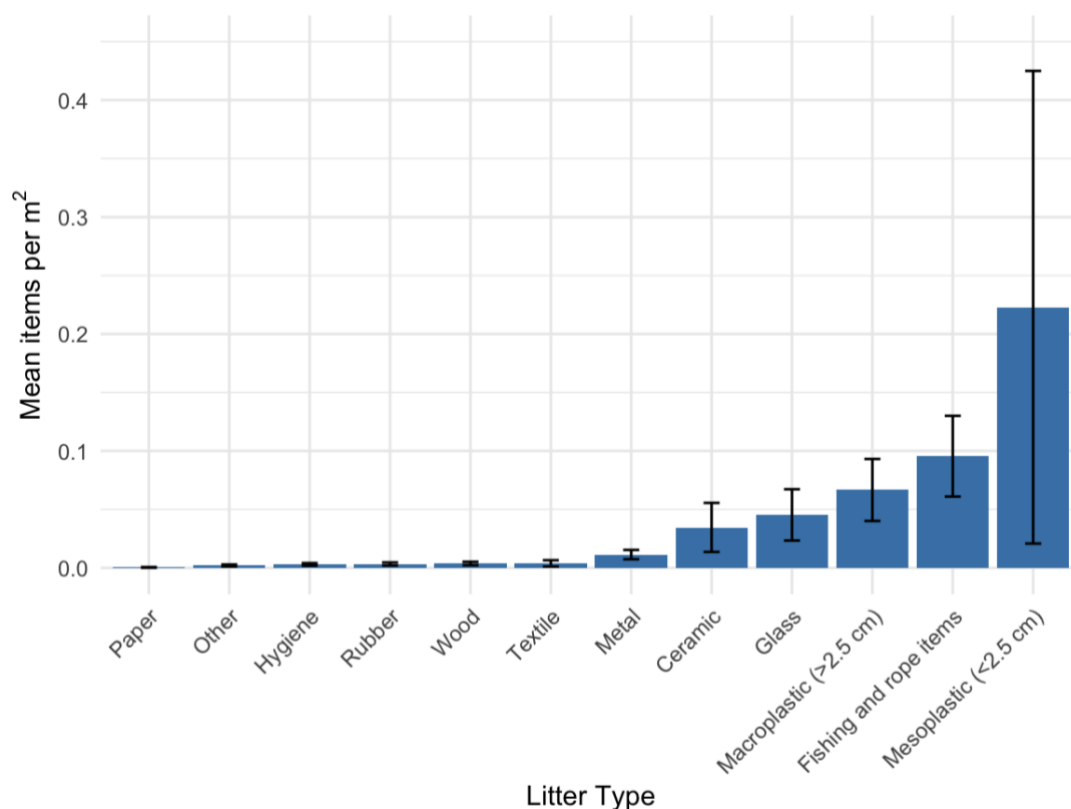
4.4.2 Material abundance reported across both transect approaches

Among material categories, mesoplastic fragments were the most abundant, with a mean of 0.22 ± 1.28 items/ m^2 across parallel transects and 0.14 ± 0.72 items/ m^2 across perpendicular transects (Figure 4.6). This was followed by fishing gear and rope related items, which comprised 36% of total macrolitter in parallel transects (0.09 ± 0.21 items/ m^2) and 51% in perpendicular transects (0.09 ± 0.32 items/ m^2).

Macroplastic was the next most abundant category, comprising 24% of macrolitter in parallel transects (0.06 ± 0.16 items/m²) and 18% in perpendicular transects (0.04 ± 0.06 items/m²). Other notable material categories included glass (0.04 ± 0.13 items/m² and 0.04 ± 0.11 items/m², respectively), ceramic (0.03 ± 0.12 items/m² and 0.02 ± 0.06 items/m², respectively), and metal (0.01 ± 0.02 items/m² in both methods).

While overall material category rankings remained consistent between transect methods, wood ranked higher in parallel transects. A full breakdown of material category values is provided in Appendix III, Table 2.

a.



b.

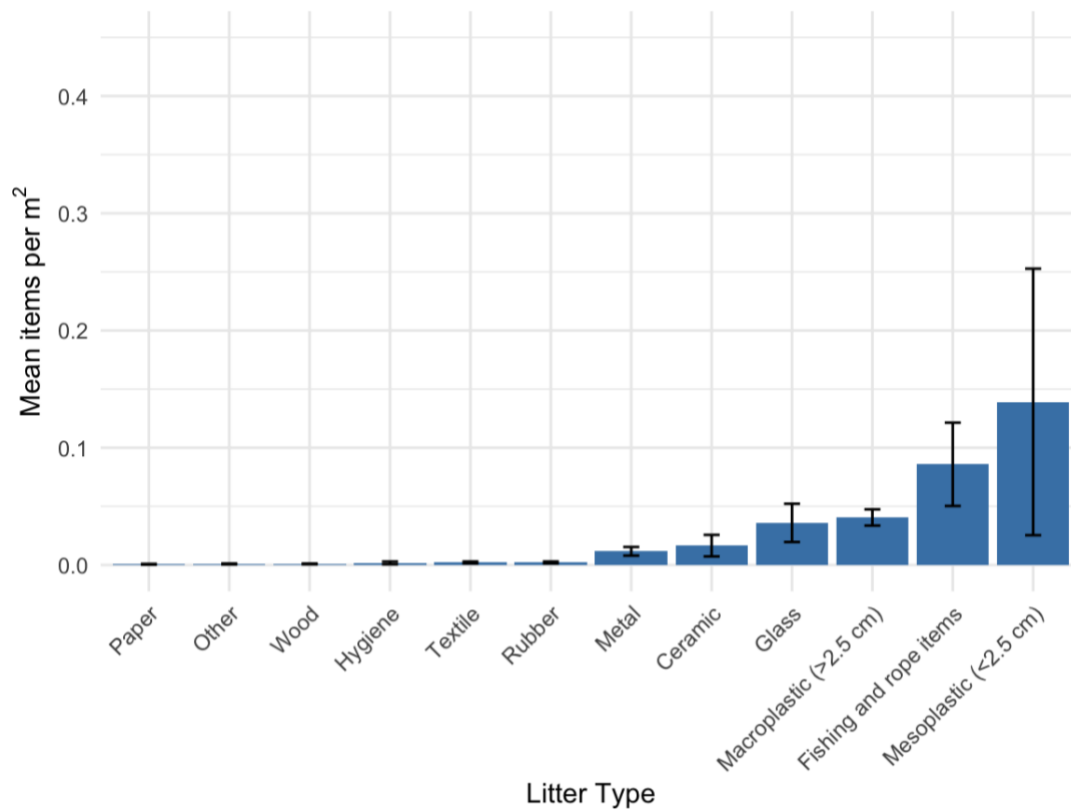


Figure 4.6: Bar charts demonstrating the mean litter abundance (items per m²) and standard error (SE) for the material categories across a) the parallel transects and b) the perpendicular transects for all litter documented across beach survey sites (N = 40).

A pairwise comparison of mean material category abundance across sites revealed no significant differences between transect methods (Figure 4.7, Appendix III Table 3). Similarly, PERMANOVA was applied (using Euclidean distance) to assess relative material abundance and detected no significant difference between transect approaches ($F_{1,78} = 0.39$, $r^2 = 0.01$, $p = 0.74$). Although differences were minor, SIMPER analysis identified fishing and rope items (30%) as the largest contributor to variation, followed by macroplastics (19%), mesoplastic (16%), and glass (15%).

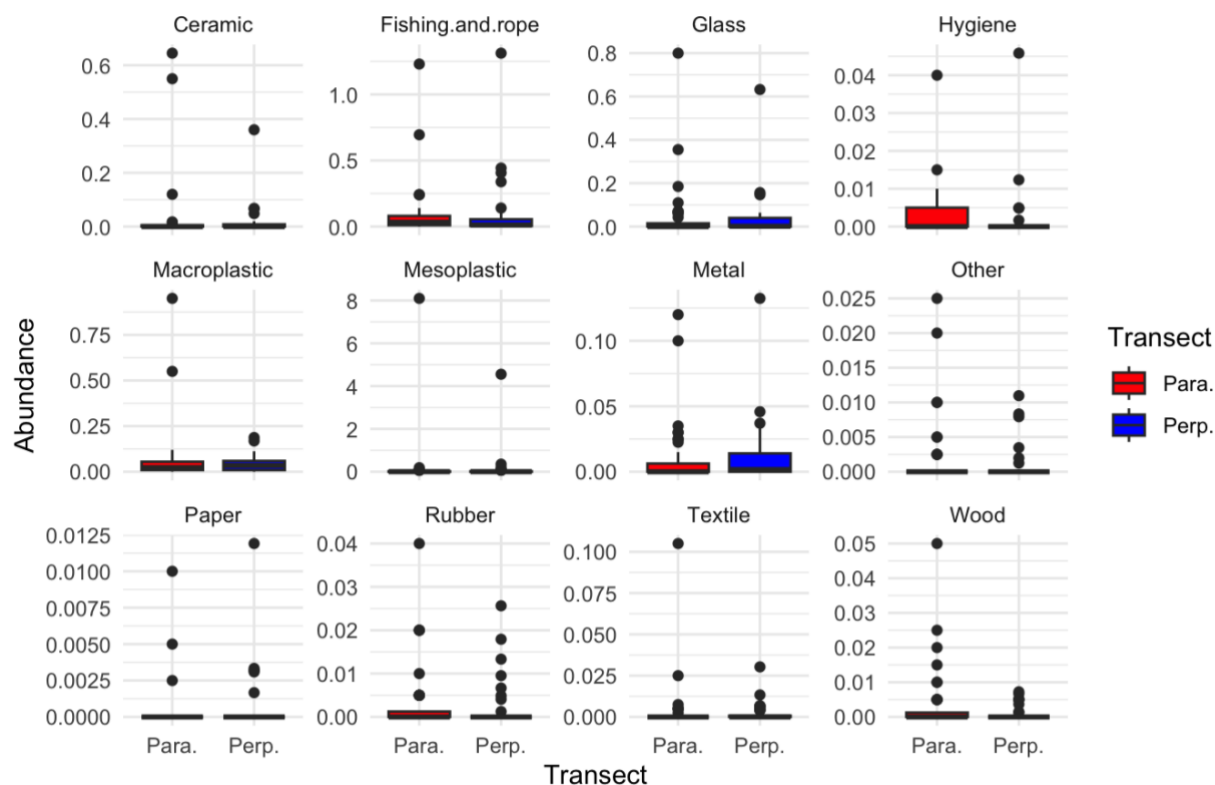


Figure 4.7: Pairwise comparison showing mean abundance (items per m^2) of beach litter material groups from parallel and perpendicular transect surveys across beach survey sites ($N = 40$).

4.4.3 Relative litter composition across both transect approaches

Principal coordinate analysis (PCoA) on Hellinger-transformed data was conducted to enable a detailed comparison of material category abundance across survey sites for both transect approaches (Figure 4.8). Vector arrows indicate the significant material categories driving site composition. Material categories and their coefficients with principal coordinates are detailed in Appendix III, Table 4. While most material categories exhibited similar influences across methods, hygiene significantly differentiated sites in the parallel transects, while metal waste did so in the perpendicular transects.

For parallel transects, the first principal coordinate (PCo1) explained approximately 50% of the variance, with high loadings for glass (1.44) and ceramic (1.02) driving differences among sites (Figure 4.8a). In contrast, the perpendicular transects yielded a lower PCo1 variance (36%), with glass (0.71) and ceramic (0.06) exerting a less

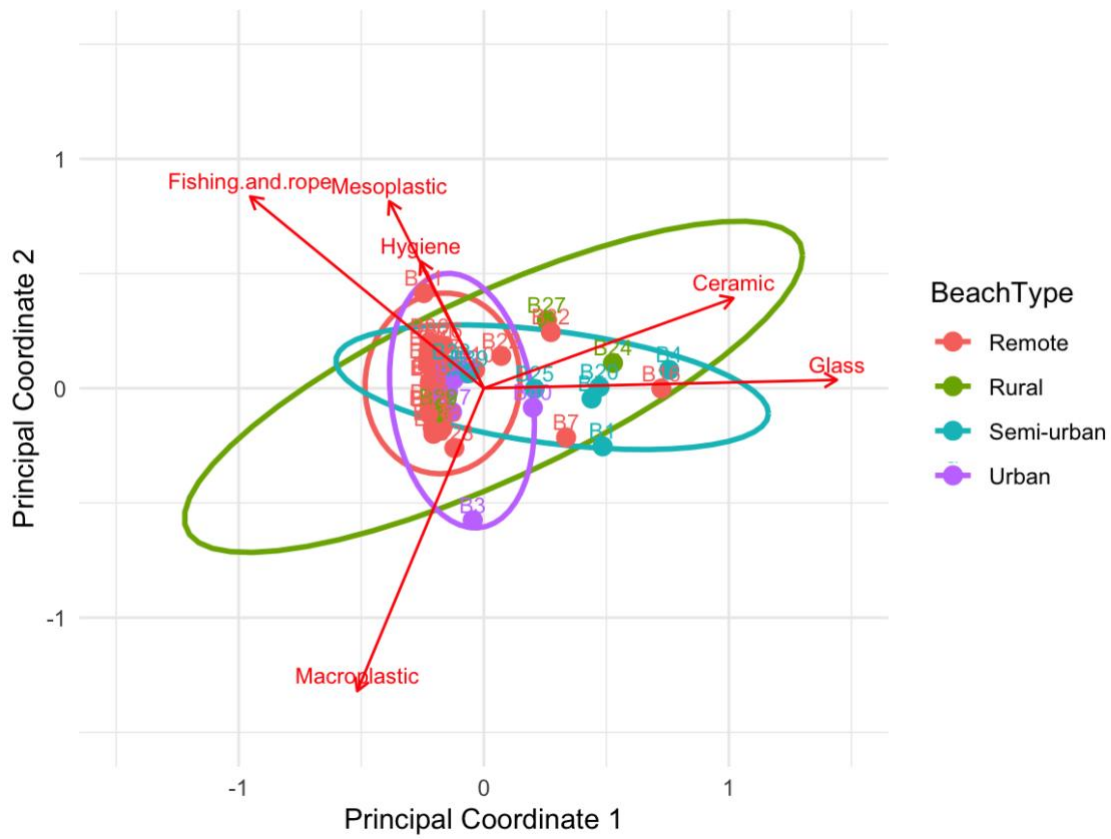
pronounced influence on this axis and a stronger one on PCo2 (-0.5 and -0.63, respectively) (Figure 4.8b).

PCo2 explained approximately 19% of the variance in the parallel transects and 25% in the perpendicular transects. In the parallel transects, macroplastic exhibited a moderate negative loading PCo1 (-0.51) and a strong negative loading on PCo2 (-1.32), while fishing-related debris showed a stronger negative loading on PCo1 (-0.95) but a positive loading on PCo2 (0.84). In the perpendicular transects, macroplastic was nearly neutral on PCo1 (-0.05) and moderately positive on PCo2 (0.77), whereas fishing-related debris had a moderate negative loading on PCo1 (-0.82) and a negative association with PCo2 (-0.39). Additionally, macroplastic and mesoplastic exhibited a similar spatial or compositional trend in the perpendicular transects, but demonstrated an inverse trend in the parallel transects.

Clustering patterns based on beach type revealed distinct grouping tendencies. Urban and remote beaches formed central clusters, in both transect approaches, indicating relatively consistent litter compositions (Figure 4.8). In contrast, rural and semi-urban sites exhibited greater variation along PCo1, correlating with glass, ceramic, and metal abundance.

A Bray-Curtis PERMANOVA found no significant difference in relative litter composition between parallel and perpendicular transect methods ($F_{1,78} = 0.65$, $p = 0.75$). Furthermore, transect method explained <1% of the variance in the Bray-Curtis dissimilarity, indicating that transect orientation had minimal impact on material composition patterns. However, PERMANOVA detected a significant difference in litter composition between beach types for the parallel transects ($F_{3,39} = 3.18$, $p = <0.01$), with beach type explaining 21% of the variance. A similar trend was observed in the perpendicular transects ($F_{3,38} = 1.73$, $p = <0.05$), though this model was weaker, explaining only 13% of the variance.

a.



b.

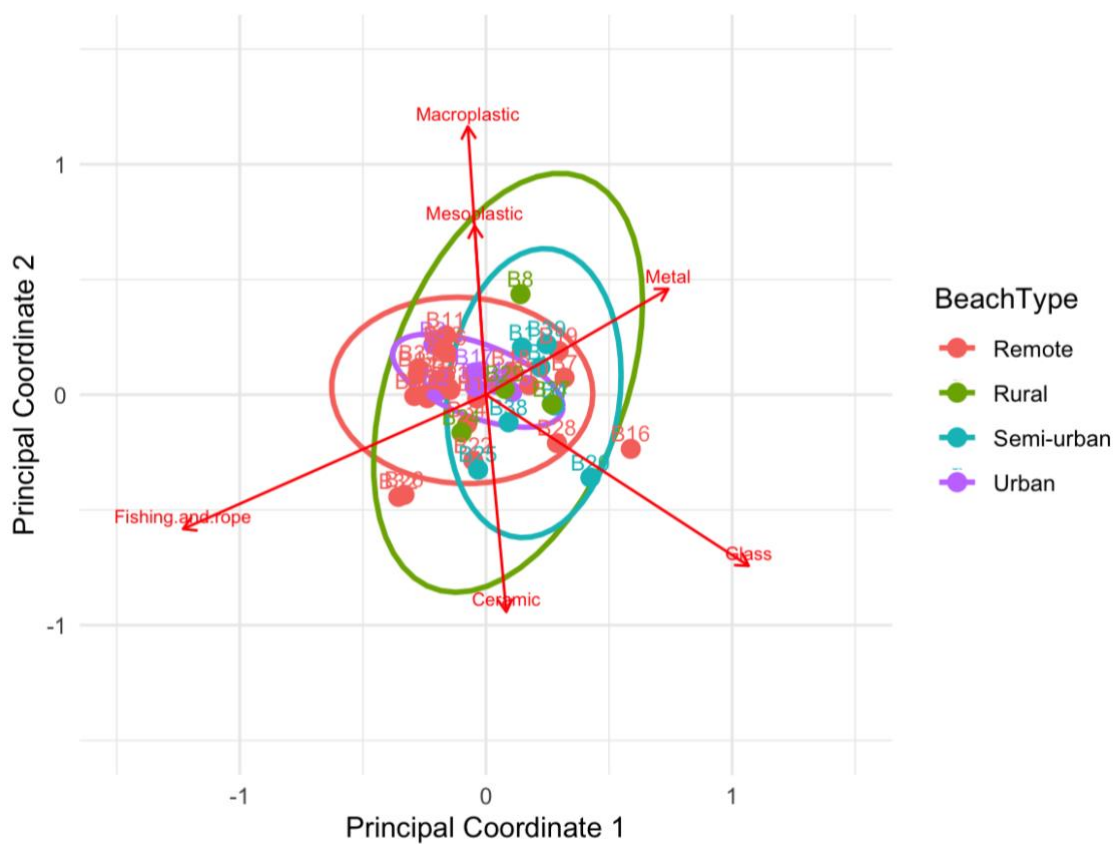


Figure 4.8: Principal Coordinate Analysis (PCoA) ordination and vector arrows relating to relative litter composition for a) parallel transect data and b) perpendicular transect data, grouped by

beach type. Mean abundance data was transformed using the Hellinger transformation method and plotted on the ordination (numerically labelled B1-B40, detailed in Appendix III, Table 1), where the distance between points reflects their dissimilarity. The direction and magnitude of vector arrows indicate the significance of material categories on clustering patterns.

4.4.4 Relationship between material abundance and site variables

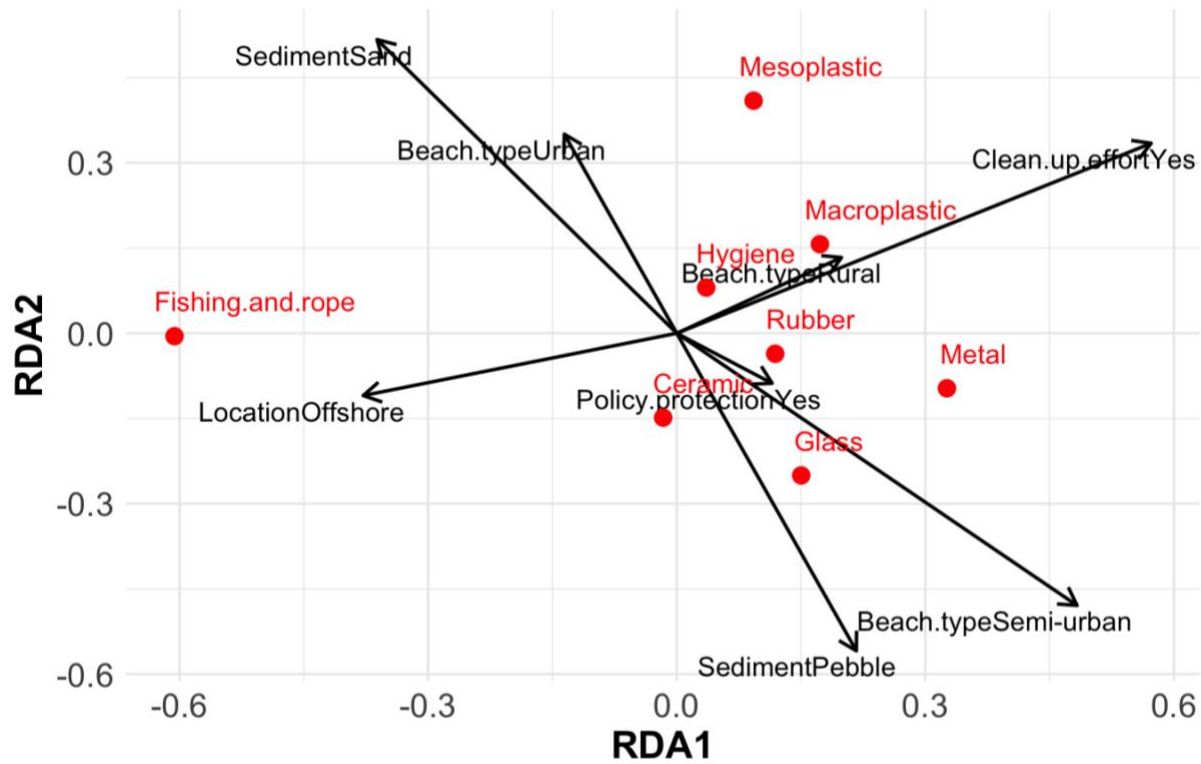
A Redundancy Analysis (RDA) model on log transformed data examined the relationship between the most abundant materials and site-related variables, with agreeable trends across both transect types (Figure 4.9).

For parallel transects, location ($F_{1,30} = 3.98$ $p = 0.05$), beach type ($F_{3,30} = 2.04$, $p = 0.05$), and sediment ($F_{2,30} = 1.25$, $p = 0.03$) were identified as significant predictors of material composition. Clean-up effort showed the highest significance ($F_{1,30} = 2.17$, $p = <0.01$), while policy protection showed no clear effect ($F_{1,30} = 0.89$, $p = 0.11$). Regarding significance of the RDA axes, RDA 1 was significant ($F_{1,30} = 7.96$, $p = <0.01$), suggesting it captures some structured variation, but most variation remains unexplained by the model.

For perpendicular transects, location ($F_{1,30} = 1.98$ $p = 0.05$), beach type ($F_{3,30} = 1.62$, $p = 0.05$), sediment ($F_{2,30} = 1.83$, $p = 0.02$) and clean-up effort ($F_{1,30} = 4.45$, $p = <0.01$) also indicated significance, while policy protection again showed no clear effect ($F_{1,30} = 1.55$, $p = 0.11$). Regarding significance of the RDA axes, RDA 1 was also significant ($F = 8.74$, $p = <0.01$). RDA model outputs are detailed in Appendix III, Tables 5 and 6.

Similar trends were observed in the biplot outputs for both transect approaches, with fishing and rope associated with offshore sites and the absence of clean-up effort, while macroplastic appeared prevalent even at sites with no active clean-up effort (Figure 4.9). Additionally, glass and ceramic correlated with semi-urban, pebble beach sites, across both transect approaches.

a.



b.

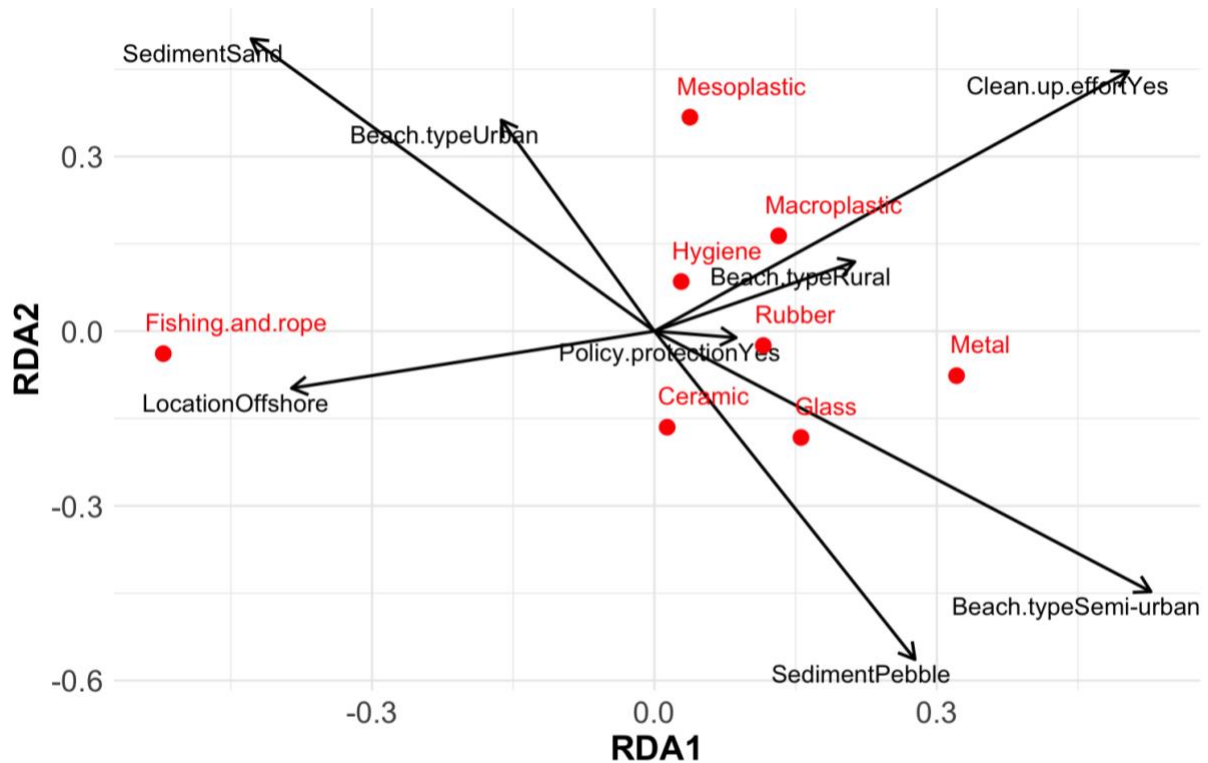


Figure 4.9: Redundancy Analysis (RDA) model and biplot illustrating relationships between material abundance (items/m²) from beach surveys (N = 40) and site variables for a) parallel and b) perpendicular transect data.

Both transect approaches identified rural sites as having the highest litter density across beach type (Table 4.2). However, uneven sample sizes within these groups may influence these rankings and other findings.

Additionally, parallel transects recorded higher average macrolitter load on mainland beaches (0.37 ± 0.58 items/m²) compared to offshore sites (0.22 ± 0.40 items/m²). Perpendicular transects showed a similar pattern, with lower litter loads on offshore sites (0.16 ± 0.45 items/m²) than mainland sites (0.27 ± 0.37 items/m²).

Table 4.2: Macrolitter abundance (mean items/m²) across site variables for parallel and perpendicular transect approaches for beach survey sites.

Site variable	N	Abundance (items m ²)	
		Parallel (Mean ± SD)	Perpendicular (Mean ± SD)
Beach Type			
Remote	23	0.24 ± 0.38	0.09 ± 0.15
Rural	4	0.39 ± 0.71	1.00 ± 1.25
Semi-urban	7	0.15 ± 0.23	0.24 ± 0.33
Urban	6	0.36 ± 0.71	0.19 ± 0.23
Location			
Mainland	13	0.37 ± 0.58	0.27 ± 0.37
Offshore	27	0.22 ± 0.40	0.16 ± 0.45
Substrate			
Pebble	14	0.56 ± 0.70	0.41 ± 0.69
Mixed	3	0.23 ± 0.16	0.1 ± 0.06
Sand	23	0.10 ± 0.09	0.09 ± 0.11
Clean-up Effort			
No Clean-up	11	0.37 ± 0.50	0.39 ± 0.73
Clean-up	29	0.23 ± 0.45	0.11 ± 0.14
Policy Protection			
No Protection	14	0.21 ± 0.31	0.20 ± 0.34
With Protection	26	0.30 ± 0.53	0.19 ± 0.47

Pebble beaches exhibited higher mean macrolitter abundance than sand beaches across both transect types. Parallel surveys reported that pebble beaches had substantially more macrolitter abundance than sand beaches, with estimates of 0.56 ± 0.70 items/m² and 0.10 ± 0.09 items/m², respectively. This pattern was also reflected in perpendicular surveys (0.41 ± 0.69 items/m² for pebble beaches compared to 0.09 ± 0.11 items/m² for sand beaches).

Clean-up efforts were associated with lower densities. In parallel surveys, sites with no clean-up efforts exhibited higher litter densities (0.37 ± 0.50 items/m²) compared to those with known clean-up efforts (0.23 ± 0.45 items/m²). This trend was even more pronounced in the perpendicular surveys (0.11 ± 0.14 items/m² and 0.39 ± 0.73 items/m², respectively).

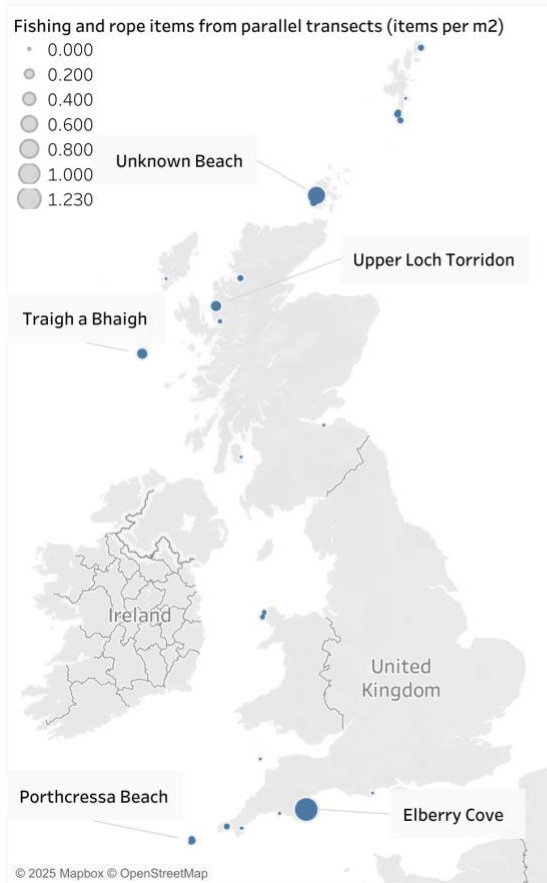
Beaches with policy protection reported a weak trend across transect approaches. In parallel surveys, beaches in areas with a level of policy protection reported a slightly higher average litter load of 0.30 ± 0.53 items/m² than unprotected sites (0.21 ± 0.31 items/m²). However, perpendicular surveys indicated a similar litter load in protected and unprotected sites (0.20 ± 0.34 and 0.19 ± 0.47 items/m², respectively).

4.4.5 Most abundant materials and items

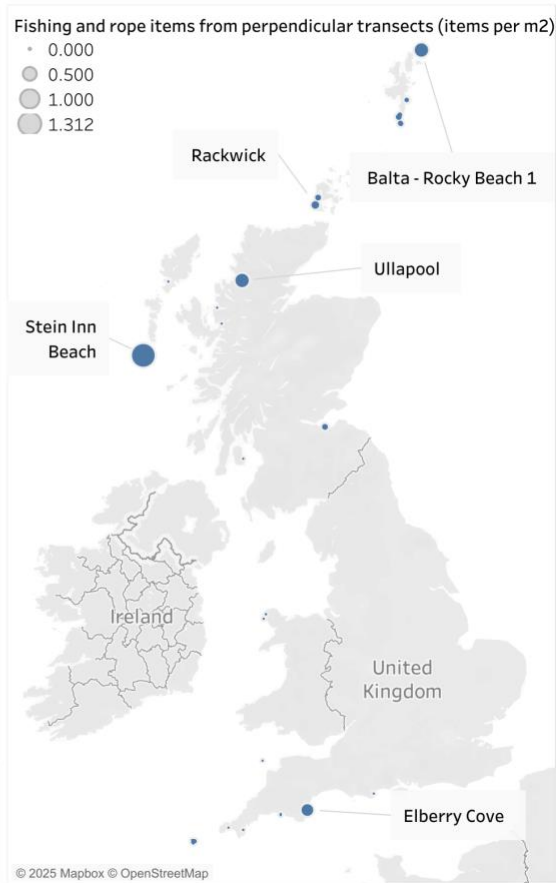
Fishing and rope related items emerged as the most prevalent material group across both survey approaches transects, accounting for 36% and 51% of the total litter recorded on parallel and perpendicular transects, respectively (Figure 4.10a and 4.10b). Parallel transects revealed string/rope, fishing net and pieces, and fishing line as the most abundant fishing and rope items (Table 4.3). These items were also among the most abundant items in parallel transects. Macroplastic was the second most prevalent material category across both transect approaches, accounting for 24% and 18% of the total litter recorded on parallel and perpendicular transects, respectively (Figure 4.10c and 4.10d).

A total of 75 different categories of litter were identified. Although the twenty most abundant items recorded across transect approaches differ slightly (Table 4.3), a Spearman's Rank correlation ($r_s = 0.64$, $n = 20$, $p = <0.01$) indicated higher-ranked items in parallel transects tended to also have higher rankings in perpendicular transects. A Wilcoxon Signed Rank test reports a borderline result ($V = 55$, $p = >0.05$), indicating no strong statistical difference between transects.

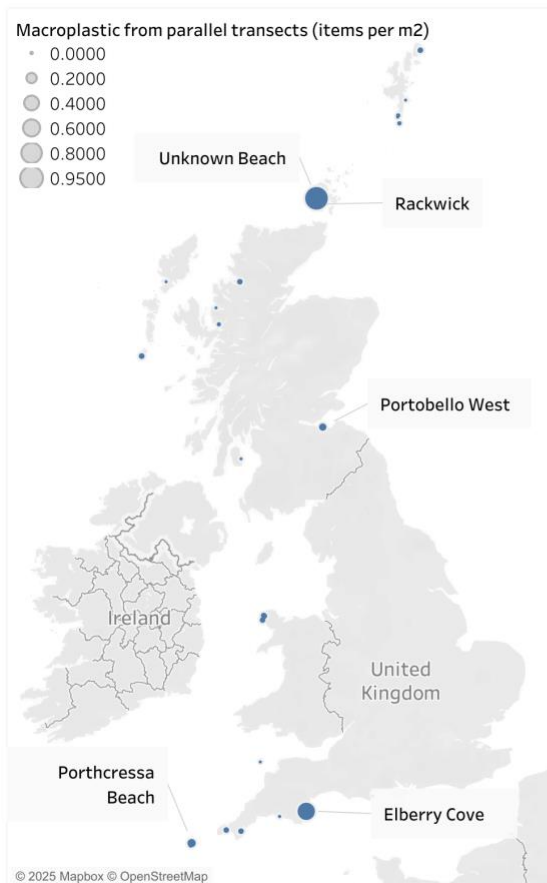
a.



b.



c.



d.

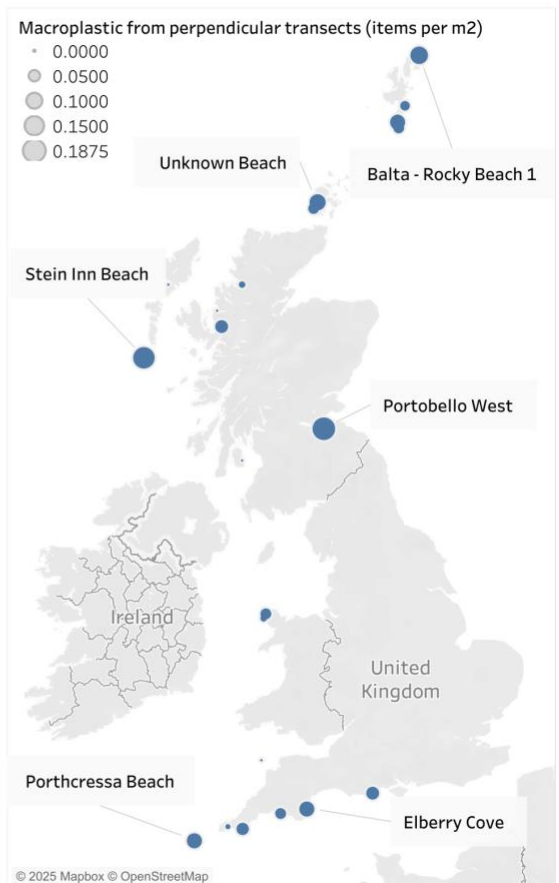


Figure 4.10: Maps of the average abundance of a) macroplastic from parallel transects, b) macroplastic from perpendicular transects, c) fishing and rope items from parallel transects, and d) fishing and rope items from perpendicular transects (items m²) for each beach survey site (N = 40). The five sites with the highest reported abundances are labelled.

Table 4.3: Most abundant items recorded on beach litter surveys (N = 40) around the coast of the UK for parallel transect data and perpendicular transect data.

Most abundant items					
Parallel transects	Items m ²	SD	Perpendicular transects	Items m ²	SD
Plastic (<2.5cm)	0.19	1.19	Plastic (<2.5cm)	0.12	1.17
Glass	0.05	0.14	Fishing net & pieces (0-50cm)	0.04	0.25
Plastic (2.5-50cm)	0.03	0.10	Glass	0.04	0.11
String/rope (0-1cm)	0.03	0.04	String/rope (0-1cm)	0.02	0.06
String/rope (>1cm)	0.02	0.07	Plastic (2.5-50cm)	0.02	0.04
Tangled string	0.02	0.11	String/rope (>1cm)	0.01	0.05
Ceramic	0.02	0.07	Ceramic	0.01	0.05
Fishing net & pieces (0-50cm)	0.02	0.06	Fishing line (angling)	0.01	0.04
Construction material	0.02	0.07	Tangled string	0.004	0.02
Plastic caps/ lids	0.01	0.04	Metal (<50cm)	0.003	0.02
Fishing line (angling)	0.01	0.04	Plastic caps/ lids	0.003	0.01
Crisp/sweet packets/lolly sticks	0.004	0.01	Crisp/sweet packets/lolly sticks	0.003	0.02
Clothing	0.004	0.02	Cigarette Butt	0.002	0.02
Metal (<50cm)	0.003	0.02	Plastic bags (e.g. shopping)	0.002	0.01
Cutlery/straws	0.003	0.01	Strapping band	0.002	0.02
Packaging/plastic sheeting	0.002	0.01	Plastic (>50cm)	0.002	0.01
Wood (0-50cm)	0.002	0.01	Construction material	0.002	0.01
Cable	0.002	0.01	Plastic bottles/containers: drinks	0.001	0.01
Plastic bags (e.g. shopping)	0.002	0.004	Fishing net & pieces (>50cm)	0.001	0.01
Foam/sponge/insulation	0.002	0.004	Containers: food (inc. fast food)	0.001	0.01
Plastic bottles/containers: drinks	0.002	0.003	Rubber fragment	0.001	0.01

4.5 Discussion

Despite the offshore and remote nature of most survey sites, along with ongoing clean-up operations and environmental protection efforts, litter was documented at every location. Across both transect approaches, litter abundance and relative material composition were broadly consistent at both national and site levels. However, data variability increased with higher mean litter abundance, and differences in material composition patterns were also evident between transect methods. These variations suggest that transect methods are more comparable in areas with lower litter loads, while certain material categories were captured more effectively by one method over the other, depending on beach morphology, sediment type, and material characteristics.

4.5.1 Comparison of litter data between transect approaches

4.5.1.1 Abundance-specific observations

Both approaches recorded comparable total litter abundances, suggesting they are both methodologically robust for assessing beach litter abundance. Parallel transects recorded 2,352 macrolitter and 1,793 mesoplastic items, while perpendicular transects recorded 1,995 macrolitter and 821 mesoplastic items. The differences can be attributed, in part, to the larger survey area covered by perpendicular transects (11,436 m²) compared to parallel transects (9,100 m²). However, when standardised by area, mean litter densities remained similar, both when including mesoplastics ($t_{1,39} = 1.34, p = 0.19$) and excluding them ($t_{1,39} = 1.34, p = 0.19$).

The higher variability in macrolitter densities within parallel transects, as reflected by their higher sum of squares ($ss_{Total} = 8.43$) and mean square ($MS = 0.22$) values, suggests that parallel surveys may capture greater spatial heterogeneity in litter abundance compared to perpendicular surveys ($ss_{Total} = 4.39, MS = 0.11$).

Despite these differences, statistical analyses reported no significant variation in litter abundance between the two transect approaches, regardless of mesoplastic inclusion mesoplastics ($t_{1,39} = 1.34, p = 0.19$). At the site level, a strong positive correlation ($r^2 = 0.75, p < 0.01$) was observed between parallel and perpendicular transect litter counts when mesoplastics were included, though the correlation was weaker ($r^2 = 0.50, p < 0.01$) when mesoplastics were excluded.

Similarly, Procrustes analysis reported a moderate similarity when mesoplastics were excluded ($m^2 = 0.50, r^2 = 0.70, p = < 0.01$) and a stronger alignment when mesoplastics were included ($m^2 = 0.25, r^2 = 0.86, p = < 0.01$), suggesting that mesoplastic abundance was consistently captured across methods.

At the site level, hierarchical clustering (AHC) consistently identified major clusters and high litter sites, though with some variability. Both approaches identified Plockton beach as having the highest litter abundance when mesoplastic was included. However, when mesoplastic was excluded, parallel transects identified Elberry Cove and an unknown

beach at Balta as the most polluted, whereas perpendicular transects ranked Plockton and Stein Inn beach as the most polluted. The consistency of identifying Plockton and Stein Inn beach as high litter sites aligns with their pebble sediment, exposure and lack of formalised clean-up efforts (Compa et al., 2022; Weideman et al., 2020), supporting the dominance of site-specific drivers in litter abundance rather than transect methodology.

Mesoplastics were the most abundant litter type across both transect approaches (0.22 ± 1.28 items/m² in parallel vs. 0.14 ± 0.72 items/m² in perpendicular transects), followed by fishing and rope-related items, which accounted for 36% of total macrolitter in parallel transects (0.09 ± 0.21 items/m²) and 51% in perpendicular transects (0.09 ± 0.32 items/m²). Macroplastic was also prominent, comprising 24% of macrolitter in parallel transects (0.06 ± 0.16 items/m²) and 18% in perpendicular transects (0.04 ± 0.06 items/m²).

Pairwise comparisons detected no significant differences in mean material category abundances across sites (Appendix III, Table 3), and PERMANOVA confirmed no significant difference in relative material abundance between transect approaches ($F_{1,78} = 0.39$, $r^2 = 0.01$, $p = 0.74$).

Despite slight differences in the most abundant items between transect approaches, a Spearman's Rank correlation ($r_s = 0.64$, $p = <0.01$) suggested higher-ranked items in parallel transects generally aligned with those in perpendicular transects. Additionally, borderline significant, a Wilcoxon Signed Rank test ($V = 55$, $p = >0.05$) indicated no significant difference in ranking the most abundant litter items between methods.

4.5.1.1 Material composition-specific observations

Principal Coordinate Analysis (PCoA) demonstrated a broad agreement in the relative composition of litter materials recorded across both transect orientations, reinforcing the robustness of both approaches for assessing beach litter abundance and composition. Glass and ceramic materials accounted for most variation in parallel transects, followed by fishing and macroplastic, while glass and fishing related debris were the main contributors in perpendicular transects, followed by macroplastic and

ceramic (Appendix III, Table 5). However, notable discrepancies emerged, with some materials exhibiting opposing trends, suggesting that certain material types accumulate differently depending on transect orientation.

Overall, fishing and rope related items consistently influenced both PCo1 and PCo2 across transect approaches, highlighting their key role in shaping compositional variability. Although both methods consistently identify fishing and rope related debris as a major driver, with negative loadings on PCo1, the differences in macroplastic and fishing debris loadings between methods indicate that each transect orientation captures distinct aspects of spatial and environmental variability affecting litter composition. Additionally, while macroplastic and mesoplastic exhibited similar trends in the perpendicular transects, they showed opposing trends in the parallel transects.

These findings suggest these materials accumulate differently in different environments and/or are influenced by distinct transport mechanisms. Fishing and rope debris are likely transported by offshore currents, accumulating at sites where marine influences and sediment dynamics favour the retention of heavier, marine sourced materials (Canals et al., 2021). In contrast, macroplastics may be more influenced by terrestrial inputs or wind-driven deposition, leading to different spatial distribution patterns (Hanke et al., 2019).

Site clustering analysis emphasises the influence of location-specific factors in shaping litter composition. Both transect approaches showed more variability in glass and ceramic accumulation at semi-urban and rural sites, while urban and remote sites demonstrated more consistent litter compositions. PERMANOVA results indicated that beach type had a greater impact on litter composition than transect orientation, with beach type explaining significantly more variation (parallel: $F_{3,39} = 3.18$, $p = <0.01$, perpendicular: $F_{3,38} = 1.73$, $p = <0.05$) compared to transect method ($F_{1,78} = 0.65$, $p = 0.75$). However, these results should be interpreted with caution due to uneven sample sizes across beach types and other categorical variables.

Redundancy analysis (RDA) further supported the alignment between both transect approaches in relation to site characteristics. Both methods consistently identified higher litter loads on pebble beaches, aligning with sediment dynamic theories (Compa

et al., 2022; Weideman et al., 2020). Additionally, both transect approaches highlighted fishing and rope related debris were strongly correlated with offshore sites, as well as the positive impact of clean-up efforts in reducing litter loads. The most prevalent litter items were also ranked similarly across methods, further demonstrating methodological comparability.

Although there were limitations related to site selection, which affected balanced sample sizes across categorical variables, this study found strong comparability between parallel and perpendicular transect approaches for assessing beach litter abundance and composition at a national level. Differences observed at a site level highlight the role of local beach dynamics and the importance of methodological considerations, including transect orientation, when interpreting abundance and compositional data.

4.5.2 Key limitations and future research recommendations

4.5.2.1: Methodological considerations and survey design

Selecting an appropriate survey method for marine litter assessments depends on the specific research or policy objectives, such as whether the focus is on freshly deposited tidal litter, accumulated debris, both, or specific items or material groups. In this study, two distinct transect orientations were applied to evaluate their comparability and suitability to monitoring beach litter abundance and composition at both site-specific and national scales. Each approach offers its own set of advantages and limitations (Table 4.4).

Table 4.4: Specific advantages and limitations of each survey transect approach as applied in this UK beach litter study (N = 40).

Method	Advantages	Limitations
Parallel transects (strandline-focused)	Covers a large area of the beach, capturing debris accumulation hotspots, especially where items accumulate in seaweed or algae.	May overestimate litter levels, as strandlines concentrate debris, while other areas of the beach may be relatively clean. Conversely, litter buried in seaweed or debris may be missed, leading to underestimates.
Perpendicular transects	Provides a cross-section of the entire beach, capturing both fresh and accumulated litter. Ensures representative sampling across different beach zones. More comparable to surveys in other environments (e.g., riverbanks, roadsides, and other coastal areas).	More time-consuming, especially when setting up multiple transects, requiring standardised placement to ensure comparability across different beaches.

One key limitation was the predominance of remote sites across survey sites, with relatively few locations near densely populated areas, which may have underrepresented anthropogenic litter contributions from urban sources. Additionally, the influence of regular clean-up efforts will have affected litter densities. OSPAR guidelines (2021) recommend that monitoring beaches should “ideally not be subject to other collection activities” to minimize such biases. With 75% of sites in this study known to have undergone clean-up activities, the reliability of the results are likely compromised.

Furthermore, time constraints limited the overall survey area, restricting direct comparisons with datasets that cover the entire beach width. Future studies comparing transect methods should aim to expand survey coverage so that parallel transects encompass the entire width of the beach. Where feasible, environmental compartments, such as above high tide, below high tide, the strandline, and a 2m

compartment at back of the beach, should be defined within transects to allow for a more comprehensive assessment of litter distribution and alignment with other survey methodologies. Similarly, perpendicular transects would benefit from additional replicates and a comparable compartmentalisation approach.

4.5.2.2 Site selection and future research considerations

Site selection for beach litter surveys should consider environmental factors such as slope, substrate, accessibility, and frequency of clean-up activities (GESAMP, 2019; OSPAR, 2021). Although resource constraints often limit identification of representative sites, employing GIS-based analysis or evaluating a broader range of beaches can enhance site selection and improve representativeness (Lavelle et al., 2024; Lee et al., 2022).

Regardless of method used, it is essential to consider site characteristics during site selection:

- Evaluate topography, shape, slope and positioning relative to wind and currents, selecting sites carefully on beaches with uneven litter distribution.
- Litter composition can include small fragments, such as polystyrene beads, which can be easily overlooked with visual counts. While collecting and hand sorting can improve accuracy, this approach may not be feasible for rapid assessments.
- Establishing clear survey boundaries is challenging on beaches without distinct strandlines or where sands merge into mudflats. Accurate and safe boundary setting is crucial.
- Standardising survey methods and litter quantification is vital for creating truly comparable, long-term datasets.

To strengthen marine litter monitoring and mitigation efforts, future research should also focus on:

- Expanding survey coverage to include a more balanced representation of remote, urban, and high-risk sites (e.g. near aquaculture zones, shipping lanes, and river outflows).
- Improving harmonisation of litter categories and measurement units reported across monitoring programs to enhance comparability between datasets at national, regional, and global scales.
- Advancing source attribution techniques to better distinguish between land-based and sea-based sources, particularly differentiating between capture fisheries and mariculture debris.
- Investigating the long-term influence of extreme weather events and seasonal fluctuations of marine litter distribution and accumulation patterns.
- Developing policy-aligned monitoring strategies that support regional and international efforts.

4.5.2.3 Improving source attribution and underlying origins of fishing-related debris

A more refined categorisation of litter items is needed to accurately trace fishing and rope-related pollution sources. For example, research suggests that OSPAR categorisation underestimates mariculture-related litter by a factor of four (Skirtun et al., 2022). Although methods exist to trace litter back to its sources, they require accurate baseline data on the relative contributions of different activities, which is challenging, particularly in distinguishing between fishing gear from capture fisheries versus mariculture debris (Pierard et al., 2022; van Duinen et al., 2022). While global estimates of marine litter sources exist, regional variations can be substantial, requiring localised studies to improve accuracy of source attribution (Morales-Caselles et al., 2021).

One promising approach involves tracking fishing activity using automatic identification systems (AIS), which could help correlate litter accumulation with fishing intensity in specific regions (Kroodsmas et al., 2018). While fishing intensity influences the presence of abandoned, lost or otherwise discarded fishing gear (ALDFG) observed, its predictive power is reduced by differences in gear technology, environmental conditions, and management conditions (Kuczenski et al., 2022; Richardson et al., 2022).

Although our understanding plastic emissions from mariculture remains limited, quantifying these contributions is essential for developing targeted mitigation strategies. In addition to improving source attribution methodologies, efforts should focus on reducing plastic leakage at its origin through enhanced waste management strategies, gear recovery programs, and industry regulations (Richardson et al., 2022).

4.5.3 Comparison of litter data with other studies

4.5.3.1 Macrolitter abundance and composition

Beach macrolitter densities in this study ranged from 0.01 to 1.96 items/m², with a mean macrolitter abundance estimated at 0.28 ± 0.46 items/m² for parallel transects and 0.20 ± 0.34 items/m² for perpendicular transects. Fishing gear and rope items, which are primarily plastic-based, along with other macroplastic items, accounted for 60% of macrolitter in parallel transects and 70% in perpendicular transects. These findings align with long-term UK studies reporting plastics as the dominant type of coastal litter (52-91%) (Nelms et al., 2020a; Schulz et al., 2015; Watts et al., 2017).

Fishing and consumer plastic related items, including caps and lids from drinking bottles and sweet/crisp wrappers, were the most frequently identified across both transect approaches, consistent with national and regional findings (Addamo et al., 2017; Watts et al., 2017).

In a six-year study conducted across nine beaches in northern Cornwall, southwest England, Watts et al. (2017) reported an average litter density of 0.02 items/m²/month, with plastic and rope fragments <50 cm as the predominant materials. Although these findings align with the present study in terms of material composition, the overall macrolitter abundance recorded here is approximately one order of magnitude higher. This discrepancy may partially be attributed to the snapshot design of this present study, in contrast to the cumulative accumulation approach used by Watts et al. (2017), as well as the inclusion of some relatively high litter sites.

Notably, mainland sites in this study exhibited greater macrolitter densities (parallel: 0.37 ± 0.58 items/m², perpendicular: 0.27 ± 0.37 items/m²) compared to offshore sites

(parallel: 0.22 ± 0.40 items/m², perpendicular: 0.16 ± 0.45 items/m²). Scotland and the Scottish Isles are relatively understudied compared to England (Nelms et al., 2017, 2020a). Although some surveys have been conducted, particularly on beaches near Edinburgh, between 1996 and 2003 (Storrier et al., 2007; Velandar and Mocogni, 1999), these data are now outdated. Using various transect approaches, these studies reported litter densities between 0.01 to 6.2 items/m², attributing most litter sources to recreation and sewage-related debris.

These findings emphasises the need for long-term monitoring programs that incorporate sampling locations representative of a country's entire coastline to more accurately capture national and regional litter accumulation patterns.

4.5.3.2 Insights from the Marine Conservation Society (MCS) data

The Marine Conservation Society (MCS) has been running a UK-wide citizen science beach survey initiative for over 20 years, generating the most comprehensive marine litter dataset at a national level (Nelms et al., 2017, 2020a). However, the MCS survey methodology differs from many scientific approaches, as it records data based on the number of volunteers, survey duration, and distances covered, making direct comparisons with other studies challenging.

Between 1994 and 2018, MCS data identified beaches (N = 2,378) along the English Channel and Celtic Sea as the most polluted, while the Scottish Continental Shelf exhibited the lowest mean litter abundance (Nelms et al., 2017, 2020a). Notably, only three MCS survey sites were in the Scottish Isles, suggesting that offshore litter loads may be underestimated in national assessments, as indicated by the findings in this study. This further underscores the importance of including offshore regions in marine litter surveys to enhance representation and strengthen long-term monitoring efforts.

Although MCS data found no significant changes in total litter abundance over time, it reported a significant increase in six specific litter items, including small plastic fragments (<2.5cm) and plastic food packaging. Additionally, MCS surveys detected no significant difference in litter density between sites inside and outside a MPA, which is consistent with the observations in this study.

A notable contrast emerged at Lundy beach, where MCS data previously identified one of the highest litter abundances, whereas this study recorded it among the lowest. This shift is likely attributed to recent clean-up efforts led by local tourism management, demonstrating the potential effectiveness of targeted mitigation strategies.

4.5.4 Sources, pathways and fates

4.5.4.1 Seasonal and environmental influences on litter accumulation

Beach litter abundance and flux is influenced by multiple environmental factors, including beach morphology, wind exposure, and seasonal variability (Galgani et al., 2015). In this study, wave and wind action, particularly from south-westerly and north-westerly directions, likely played a key role in shaping litter abundance and distribution patterns. Across western Europe, winter and spring typically exhibit the highest litter levels, although large-scale seasonal patterns remain vague, in part due to data limitations (Brennan et al., 2018; Rieger et al., 2024).

Given these patterns, litter densities observed in this study may represent an underestimation, as surveys were conducted during summer months and focused primarily on remote, offshore, sandy and sheltered sites. In contrast, MCS data reported no significant seasonal effect on litter abundance (Nelms et al., 2017). The discrepancy between these findings and other studies across Europe may stem from the insufficient statistical power or variations in survey effort across datasets (Rieger et al., 2024).

Beyond seasonal factors, survey timing, meteorological, as hydrological conditions, as well as proximity to rivers, may influence short-term litter dynamics (Rieger et al., 2024). This study experienced stormy conditions during the first two weeks of surveys along the south coast, which may have temporarily influenced litter deposition patterns. Additionally, this survey was conducted shortly after the COVID-19 pandemic, a period associated with shifts in localised activities, waste generation, and management practises. Furthermore, tourism is a significant part of the UK economy, but the Office

for National Statistics (2021) estimated that, following the COVID-19 pandemic, there were 6.4 million visits to the UK in 2021, 43 % fewer than 2020.

4.5.4.2 Anthropogenic drivers of litter accumulation

Beyond environmental factors, proximity to urban centres, the intensity of fishing activity, and the concentration of shipping traffic remain key drivers of marine debris accumulation (Moriarty et al., 2016; Prevenios et al., 2018; Rieger et al., 2024). Although plastics can travel vast oceanic distances, and have been found to contribute the majority of litter in some remote areas (Bouwman et al., 2016; Ryan et al., 2019), recent studies suggest that coastal areas primarily accumulate locally sourced macroplastics (Morales-Caselles et al., 2021). As a result, the influence of distant plastics on the findings in this study is likely minimal.

Fishing gear and rope materials were the most prevalent macrolitter category, with commonly recorded items included synthetic fishing nets, lines, ropes, and trap-related debris, aligning with previous research identifying fishing gear as one of the ten most commonly found litter types on European beaches (Addamo et al., 2017). Litter densities were particularly high along the Scottish Isles and the southwest coast of England, regions known for intensive fishing activity and offshore litter inputs (Anbleyth-Evans and Williams, 2018).

Some studies estimate that sea-based sources, particularly from fishing and aquaculture related activities, may contribute up to 50% of marine plastic pollution in certain regions (Kaandorp et al., 2023; Krüger et al., 2020; Li et al., 2016; Rieger et al., 2024). These materials pose significant risks to marine life, leading to entanglement hazards that can persist in the environment for extended periods, impacting some species at the population level (Gilman et al., 2021; Pierpoint, 2000; Simmonds, 2012; Votier et al., 2011).

For example, research into turtle entanglement in similar UK coastal regions has found peak encounters occurring between July and October, coinciding with their seasonal migration to feed on jellyfish (Pierpoint, 2000). Additionally, plastic ingestion and entanglement of marine mammals (Allen et al., 2012; Kirkwood et al., 1997; Ryan et al.,

2009; Simmonds, 2012) and seabirds (Alley et al., 2022; O’Hanlon et al., 2019; Votier et al., 2011) are well documented concerns in the UK, with some studies reporting high associated mortality rates (Votier et al., 2011).

4.5.5 Management considerations

4.5.5.1 Economic and environmental costs of marine litter

Marine littering and plastic pollution is a complex problem linked to human behaviour, influenced by beach cleaning efforts, waste disposal infrastructure, and plastic regulation (Corraini et al., 2018; Jambeck et al., 2015a). In the UK alone, marine litter can incur significant socioeconomic costs. Although information is outdated, examples include the fishing industry in Scotland, which has been estimated to lose £10 million annually due to gear damage and time lost removing debris (Hastings and Potts, 2013). Additionally, UK municipalities have been estimated to spend approximately £15-16 million annually on beach litter removal, a 37% increase from the previous decade (Mouat et al., 2010).

4.5.5.2 Policy and regional monitoring considerations

Land-based management challenges, including insufficient waste infrastructure and a lack of upstream policies governing plastic products, are the fundamental drivers of plastic pollution (Galgani et al., 2015, p. 2; Morales-Caselles et al., 2021). Although regulations exist for the maritime and fisheries industries regarding waste management, the lack of adequate port reception facilities and management of sea-based sources of waste further exacerbate the issue (GESAMP, 2021). Strengthening regulatory alignment with regional and international frameworks is critical to mitigating plastic pollution impacts (UNEP, 2021a).

Effective marine litter monitoring requires standardised methodologies, seasonal assessments, and coordinated international efforts to ensure comparable and policy-relevant data. A summary of relevant of marine litter policies, monitoring frameworks,

coordination mechanisms, and data collation mechanisms for beach litter monitoring is provided in Appendix III, Table 7.

At the regional level, the EU Marine Strategy Framework Directive (MSFD) Technical Group on Marine Litter recommends seasonal beach litter assessments to account for fluctuations in litter accumulation and to identify accumulation hotspots and sources of specific litter types (Galgani et al., 2024). This includes targeted monitoring of fishing gear, riverine inputs, and extreme-weather related debris influxes to better understand litter pathways and impacts on marine ecosystems.

In addition to this, the OSPAR Commission has implemented one of the most comprehensive regional monitoring frameworks, surveying 50 indicator beaches across six North-East Atlantic regions since 1998. OSPAR's surveys use a standardised protocol, applying a 100m parallel transect from the water's edge to the back of the beach (OSPAR, 2021; Wenneker and Oosterbaan, 2010). This dataset has provided long-term insights into litter trends and served as a valuable model for international harmonisation of monitoring efforts (Rieger et al., 2024). However, such programs require substantial timelines, financial resources, and logistical coordination to ensure data robustness (Hidalgo-Ruz and Martin Thiel, 2015).

Expanding citizen science and academic initiatives can play a crucial role in addressing these challenges (Hidalgo-Ruz and Martin Thiel, 2015; Nelms et al., 2020b; Rieger et al., 2024). Engaging volunteers in large-scale, long-term beach litter surveys not only facilitates data collection in resource limited areas but also enhances public awareness and behavioural change. However, for citizen science data to be effectively integrated into scientific and policy frameworks in the UK, concerns regarding survey standardisation, data comparability, and collaboration between non-governmental organisations (NGOs) and academic institutions must be addressed through harmonised protocols and rigorous analytical methods.

At the global level, the United Nations Environment Assembly (UNEA) has recognised the escalating impact of plastics pollution and is developing an international plastics treaty to strengthen global commitments to plastic waste reduction and management (UNEP, 2023b). A dedicated subsection of the draft text focuses on fishing gear,

acknowledging its persistent contribution to marine litter. Given that beach litter is already a widely recommended environmental indicator, its integration into a global framework under UNEA could enhance international data collection and harmonisation efforts, informing pollution management strategies. Aligning regional initiatives like OSPAR and MSFD with global efforts under UNEA will be critical in developing a cohesive, science-driven approach to marine litter monitoring and mitigation.

4.5.5.3 Management of fishing and rope-related debris

In response to findings of this study, stakeholder engagement was conducted to discuss waste management challenges associated with fishing gear and rope-related litter, culminating in a policy brief (Appendix IV). Despite sustainability efforts, such as gear repair initiatives and some biodegradable alternatives, significant barriers remain due to complex supply chains, lack of monitoring, and economic constraints.

4.6 Conclusion

This rapid assessment study demonstrates that both perpendicular and parallel transect methods can effectively assess beach litter abundance, composition, and distribution across 40 UK survey sites. Overall litter patterns were similar between methods, with statistically comparable abundances (0.28 ± 0.46 items/m² for parallel transects and 0.20 ± 0.34 items/m² for perpendicular transects). Despite some variations in site rankings, material types, and clustering patterns, both methods consistently identified the most prevalent litter items and hotspots, most notably at Plockton and Stein Inn beach.

Environmental factors such as coastal morphology and sediment characteristics, as well as anthropogenic drivers like fishing activity and beach type, strongly influenced litter accumulation. While these findings align with previous studies, discrepancies in specific litter quantities likely result from differences in methodological approach, survey timing, meteorological conditions, clean-up efforts, and the post-pandemic landscape.

These results highlight the need to establish representative beach sites and adopt standardised, comparable monitoring protocols for long-term UK litter datasets. Harmonising existing approaches across national, regional, and international platforms is essential for identifying high-risk areas and materials, thereby enabling targeted mitigation strategies. Future research should integrate diverse monitoring tools, ranging from *in-situ* assessments and remote sensing to citizen science, to provide a comprehensive understanding of marine litter dynamics and support effective policymaking.

5.1 Overall conclusions

Anthropogenic pollution represents a critical concern within the framework of planetary boundaries, which delineate thresholds for sustainable human activity, crossing which would threaten global ecosystem stability and sustainability (Rockström et al., 2009). Plastics, along with chemicals, heavy metals, and nutrient inputs, contribute significantly to aquatic pollution, yet monitoring their sources, pathways, and impacts remains complex due to financial constraints, shortages of trained personnel, and the dynamic nature of aquatic environments (Förstner and Wittmann, 2012; Fouzia, 2019; GESAMP, 2021; Valdor et al., 2016; Yuan et al., 2007). These challenges are particularly pronounced in resource limited, understudied regions, where high population densities and inadequate waste management infrastructure exacerbate plastic pollution.

This study aimed to develop practical and cost-effective approaches for assessing marine macroplastic pollution and its potential ecosystem impacts. Specifically, it investigated:

- An approach to quantify the influence of surface macroplastic loads on microbial community dynamics at the base of the food web.
- The potential of mesopredatory sharks as bioindicators of anthropogenic pollution.
- The application and comparability of widely used transect methodologies for marine litter assessments.

5.1.1 Macroplastic and microbial community dynamics

The experimental investigation of macroplastic's influence on microbial communities contributes to the growing field of plastisphere research, exploring how plastic debris functions as a substrate for microbial colonisation. While this study could not identify impacts associated with plastic materials specifically as no other medium was tested, it reinforces the need for further research, given that plastic is the most abundant litter material. Existing literature suggests potential relationships between community

assemblages and plastic material types, warranting additional exploration. The cost-effective design of this study offers an accessible approach for expanding research into macroplastic pollution and its ecological implications.

Preliminary findings indicate that high macrolitter loads, especially if they have been biofouled, influence ambient plankton communities. However, a more comprehensive investigation is required to assess the long-term and cascading effects of floating macroplastic on ecosystem function. Future studies should expand the experimental scope, incorporating a wider range of relevant environmental conditions, trophic interactions, and broader ecosystem dynamics to capture the full extent of macroplastic pollution impacts.

5.1.2 Sharks as bioindicators of anthropogenic pollution

This desk-based study explored the feasibility of using mesopredatory sharks as bioindicators for anthropogenic pollution loading, particularly nitrogen-based pollutants. While this study does not directly assess the impact of plastics on sharks, it highlights potential linkages between plastic pollution, nitrogen inputs, and urbanisation, which could influence trophic interactions and ecosystem health. Given policy recommendations prioritising nitrogen and plastic as key ocean health indicators (UNEP, 2021b), this research supports the need for integrated pollution assessments.

However, significant knowledge gaps persist in understanding how urbanisation influences isotopic signatures in sharks, as well as the broader ecological consequences of anthropogenic nitrogen and plastic pollution. Future studies should explore how different pollution sources interact, affect trophic dynamics, and influence species-specific responses, particularly in urban coastal environments where plastic and nitrogen pollution are most prevalent. Expanding research beyond mesopredatory sharks to other trophic levels and sentinel species could strengthen monitoring frameworks.

5.1.3 Marine litter monitoring and data harmonisation

A rapid marine litter assessment across UK beaches evaluated the effectiveness of commonly used monitoring methodologies. The results underscored the importance of harmonising consistent data collection protocols to improve comparability across studies and policy frameworks. While the study identified the most dominant litter materials, with mesoplastics, fishing gear, rope, and macroplastics emerging as the most prevalent items, it also highlighted persistent challenges in standardising marine litter assessments.

Expanding the geographic scope of marine litter assessments, particularly in environmentally sensitive regions in Africa and Asia, would enhance global comparisons of plastic pollution trends. Strengthening harmonised methodologies and reporting standards is essential for generating robust datasets that inform policy, management, and mitigation strategies at national, regional, and global levels.

5.1.4 Challenges, gaps, and future directions

Despite extensive efforts to date, significant challenges remain in linking plastic production, consumption, and environmental leakage within a cohesive global monitoring framework. Current monitoring initiatives may be more effective at tracking plastic pollution rather than actively reducing it, highlighting the need for:

- Enhanced data integration across the plastics lifecycle, including improved classification systems and terminology to standardise global assessments.
- Expanded monitoring of plastic production and trade flows to strengthen source attribution and mitigation strategies.
- Greater harmonisation of national and global reporting mechanisms, ensuring alignment with SDG indicators, international agreements, and industry-led reporting mechanisms.
- Further investigation into the ecosystem-level impacts of plastic pollution, particularly its role in exacerbating climate change and biodiversity loss through

emissions, habitat degradation, altered biogeochemical cycles, and disruptions to trophic interactions.

Discussions surrounding an international legally binding plastics treaty underscores the urgency of developing a comprehensive and coordinated approach to tackling plastic pollution (UNEP, 2023b). This is arguably one of the most complex environmental regulations ever deliberated, and its success depends on being grounded in robust scientific evidence. Implementing a tiered approach to data collection, accommodating different levels of complexity, would improve inclusivity, accessibility, and effectiveness in integrating research findings into global databases. Supporting regional initiatives such as the Regional Seas Convention and UN SDG databases would strengthen collaborative efforts.

Prioritising research in highly impacted and ecologically vulnerable areas is essential to target conservation and management strategies where they are most needed.

Demonstrating credible progress toward international plastic pollution objectives will also be critical in securing political support, financing, and long-term policy implementation.

5.1.5 Final thoughts

This study contributes to the ongoing effort to refine methodologies for assessing marine plastic pollution, offering practical and scalable approaches that can be integrated into broader monitoring frameworks. However, greater cross-sectoral collaboration and data-sharing initiatives are needed to bridge the gap between research, policy, and mitigation efforts. Strengthening harmonised protocols and reporting metrics will enhance the transparency and effectiveness of international agreements, ensuring that monitoring efforts translate into meaningful reductions in plastic pollution.

Appendix I

Table 1: Chlorophyll a on day 1 across all microcosms (pre-treatment).

Microcosm (pre- treatment day 1)	Mean Chl.a (ug/L)	SD	SE	Shapiro-Wilk test		
				W-Statistic	Df	Sig.
100 PET clean	0.030	0.012	0.007	0.99	3	0.81
50 PET clean	0.052	0.024	0.014	0.85	3	0.25
100 PVC clean	0.020	0.004	0.002	0.99	3	0.79
50 PVC clean	0.029	0.024	0.014	0.84	3	0.22
100 PET biofilm	0.029	0.024	0.014	0.78	3	0.06
50 PET biofilm	0.036	0.024	0.014	0.78	3	0.08
100 PVC biofilm	0.046	0.024	0.014	0.78	3	0.05
50 PVC biofilm	0.047	0.024	0.014	0.87	3	0.30
Control	0.037	0.014	0.002	0.99	12	0.79

Table 2: Chlorophyll a across controls for the duration of the experiment.

Treatment	Chl.a				r ²
	Day 1	Day 2	Day 3	Day 9	
Controls	0.057	0.100	0.111	0.118	0.84
	0.056	0.066	0.092	0.100	0.96
	0.038	0.086	0.050	0.138	0.57
	0.021	0.074	0.077	0.084	0.74
	0.024	0.053	0.077	0.910	0.98
	0.021	0.076	0.099	0.142	0.98
	0.015	0.075	0.060	0.235	0.75
	0.035	0.051	0.090	0.125	0.98
	0.039	0.080	0.093	0.100	0.86
	0.041	0.144	0.172	0.314	0.94
	0.040	0.097	0.123	0.118	0.78
	0.056	0.132	0.163	0.263	0.97
Mean	3.70	8.61	10.05	15.23	
SD	1.44	2.83	3.70	7.52	
SE	0.42	0.82	1.07	2.17	

Table 3: Normality tests for cell counts across treatments.

Treatment	Average cell count (x10 ³ cells/L)				Average cell count (x10 ³ cells/L)			
	Day 1	W-Statistic	Df	Sig.	Day 7	W-Statistic	Df	Sig.
100 PET clean	243.67	0.98	3	0.73	375.00	0.97	3	0.66
50 PET clean	291.00	0.98	3	0.70	362.67	0.98	3	0.70
100 PVC clean	147.00	0.99	3	0.84	210.00	0.91	3	0.41
50 PVC clean	131.67	0.93	3	0.49	173.00	0.82	3	0.16
100 PET biofilm	68.67	0.99	3	0.80	1117.33	0.79	3	0.08
50 PET biofilm	471.00	0.97	3	0.68	2019.33	0.97	3	0.64
100 PVC biofilm	255.00	0.84	3	0.21	3012.00	0.87	3	0.31
50 PVC biofilm	299.67	0.88	3	0.34	1549.00	0.95	3	0.58
Control	306.50	0.87	12	0.07	533.75	0.87	12	0.06

Table 4: Average chlorophyll a, cell density, species richness, diversity index and evenness across treatments.

Parameter	Treatment											
	Sample day	100 PET clean	50 PET clean	100 PVC clean	50 PVC clean	100 PET biofilm	50 PET biofilm	100 PVC biofilm	50 PVC biofilm	Control		
Avg. Chl.a (ug/L)	1	0.030	0.052	0.020	0.029	0.029	0.036	0.046	0.047	0.037		
	2	0.204	0.099	0.084	0.093	3.775	4.464	3.474	1.372	0.086		
	3	0.172	0.071	0.061	0.047	0.743	2.927	3.334	2.319	0.101		
	9	0.167	0.139	0.070	0.091	2.476	3.552	2.916	2.331	0.221		
Avg. cell density (cells/L x 10 ³)	1	243.67	291.00	147.00	131.67	68.67	471.00	255.00	299.67	306.50		
	7	375.00	362.67	210.00	173.00	1117.33	2019.33	3012.00	1549.00	533.75		
Species richness	1	10	8	7	8	7	9	9	8	10		
Diversity index (H)	1	-2.1	-1.88	-1.66	-1.73	-1.14	-1.66	-1.04	-1.02	-1.7		
Evenness	1	-0.91	-0.90	-0.85	-0.83	-0.59	-0.76	-0.47	-0.49	-0.74		
Species richness	7	8	9	8	9	10	10	6	9	10		
Diversity index (H)	7	-1.59	-1.49	-1.49	-1.32	-1.37	-1.04	-0.98	-1.02	-1.58		
Evenness	7	-0.76	-0.68	-0.72	-0.60	-0.59	-0.45	-0.55	-0.46	-0.69		

Table 5: PCA correlation coefficients for taxonomic groups.

Taxonomic group	PC1	PC2
<i>Coscinodiscus spp.</i>	-0.37	-0.47
<i>Odontella spp.</i>	-0.50	-0.47
<i>Rhizosolenia spp.</i>	-0.39	-0.25
<i>Chain forming spp.</i>	-0.26	-0.85
<i>Thalassiosira spp.</i>	-0.41	-0.40
<i>Pseudo.nitzschia spp.</i>	-0.45	0.02
<i>Bacillaria spp.</i>	-0.00	-0.13
<i>Ceratium spp.</i>	-0.17	-0.32
<i>Nauplii spp.</i>	-0.16	-0.22
<i>Polychaetae spp.</i>	-0.15	0.14
<i>Navicula spp.</i>	0.83	-0.02
<i>Thalassionema spp.</i>	0.74	-0.11
<i>Prokaryote spp.</i>	0.66	-0.01

Appendix II

Table 1: Mean and standard deviation of carbon and nitrogen isotopes (‰) for mesopredatory shark samples from the CSIDP.

Species	Mean.d13C	SD.d13C	Mean.d15N	SD.d15N	Mean.d15Nc	SD.d15Nc
<i>Carcharhinus</i>						
<i>amblyrhynchos</i>	-14.01	1.15	10.81	0.54	8.19	0.54
<i>Carcharhinus brachyurus</i>	NA	NA	13.52	1.01	7.16	1.01
<i>Carcharhinus falciformis</i>	-16.41	0.54	14.21	2.25	8.23	1.07
<i>Carcharhinus limbatus</i>	-16.54	0.79	15.95	1.01	16.49	2.96
<i>Carcharhinus perezii</i>	-14.78	1.58	14.88	0.85	14.84	0.85
<i>Carcharhinus plumbeus</i>	-17.60	0.78	14.34	0.85	11.93	0.85
<i>Galeocerdo cuvier</i>	-17.00	0.82	12.35	1.15	9.17	1.12
<i>Loxodon macrorhinus</i>	-15.40	1.01	11.66	0.59	7.81	0.59
<i>Rhizoprionodon taylori</i>	-15.41	0.76	12.23	0.66	9.05	0.66
<i>Rhizoprionodon terraenovae</i>	-17.03	0.19	16.04	0.57	17.70	0.57
<i>Scoliodon laticaudus</i>	-16.53	1.54	15.41	0.76	15.81	0.76
<i>Scyliorhinus canicula</i>	-17.19	0.98	12.39	3.15	8.68	3.14
<i>Sphyrna lewini</i>	-15.66	0.87	13.69	1.54	10.43	1.86
<i>Sphyrna tiburo</i>	-16.20	1.07	14.55	2.06	15.20	3.49
<i>Sphyrna zygaena</i>	-15.90	0.28	15.48	1.21	9.72	1.79
<i>Squalus acanthias</i>	-16.91	0.65	12.97	1.20	9.35	1.16
<i>Squalus megalops</i>	-16.93	0.15	12.29	0.55	9.32	0.55
<i>Triaenodon obesus</i>	-11.98	1.61	9.64	0.77	7.02	0.77

Table 2: Mean and standard deviation for d15Nc (‰) values for mesopredatory shark species sampled from the CSIDP across each location.

Location/Species	Mean d15Nc	SD d15Nc
Aomori	8.89	0.83
<i>Squalus acanthias</i>	8.89	0.83
Balearic Island	5.45	0.64
<i>Scyliorhinus canicula</i>	5.45	0.64

CE Pacific	8.65	1.00
<i>Carcharhinus falciformis</i>	8.53	0.81
<i>Sphyrna lewini</i>	9.89	0.61
<i>Sphyrna zygaena</i>	8.56	1.26
English Channel	10.65	1.17
<i>Scyliorhinus canicula</i>	10.65	1.17
Florida	11.44	1.38
<i>Carcharhinus falciformis</i>	12.10	0.00
<i>Sphyrna lewini</i>	12.58	0.85
<i>Sphyrna tiburo</i>	10.27	0.66
Galveston	17.75	0.60
<i>Carcharhinus limbatus</i>	18.09	0.53
<i>Rhizoprionodon terraenovae</i>	17.70	0.57
<i>Sphyrna tiburo</i>	17.56	0.58
Great Barrier Reef	7.63	0.88
<i>Carcharhinus amblyrhynchos</i>	8.19	0.54
<i>Galeocerdo cuvier</i>	8.33	0.00
<i>Triaenodon obesus</i>	7.02	0.77
<i>Gulf St. Vincent</i>	7.68	1.09
<i>Carcharhinus brachyurus</i>	7.68	1.09
Hong Kong	15.81	0.76
<i>Scoliodon laticaudus</i>	15.81	0.76
KZN Nets	11.29	0.47
<i>Carcharhinus limbatus</i>	11.28	0.40
<i>Galeocerdo cuvier</i>	9.99	0.08
<i>Sphyrna lewini</i>	11.26	0.36
<i>Sphyrna zygaena</i>	11.55	0.40
Mayotte	7.52	0.71
<i>Carcharhinus falciformis</i>	7.52	0.71
New Providence	14.84	0.85
<i>Carcharhinus perezii</i>	14.84	0.85
North Sea	10.32	1.59
<i>Scyliorhinus canicula</i>	12.23	0.87
<i>Squalus acanthias</i>	9.72	1.25
Nosy Hao	8.11	0.93
<i>Carcharhinus falciformis</i>	9.66	1.75
<i>Loxodon macrorhinus</i>	7.81	0.59
<i>Sphyrna lewini</i>	8.64	1.12

Queensland	9.10	0.81
<i>Galeocerdo cuvier</i>	9.14	1.14
<i>Rhizoprionodon taylori</i>	9.05	0.66
<i>Sphyrna lewini</i>	10.55	1.65
Reunion Island	9.32	0.55
<i>Squalus megalops</i>	9.32	0.55
Spencer Gulf	6.85	0.83
<i>Carcharhinus brachyurus</i>	6.85	0.83
St Helena	11.93	0.85
<i>Carcharhinus plumbeus</i>	11.93	0.85

Table 3: Games-Howell pairwise comparison results of $\delta^{15}\text{Nc}$ (‰) between species groups.

Test #	Group 1	Group 2	Statistic	df	p-value	Significance
1	<i>Carcharhinus amblyrhynchos</i>	<i>Carcharhinus brachyurus</i>	7.12	101.55	2.40E-08	****
2	<i>Carcharhinus amblyrhynchos</i>	<i>Carcharhinus falciformis</i>	0.26	104.10	1	ns
3	<i>Carcharhinus amblyrhynchos</i>	<i>Carcharhinus limbatus</i>	18.80	50.46	0	****
4	<i>Carcharhinus amblyrhynchos</i>	<i>Carcharhinus perezii</i>	36.98	50.71	0	****
5	<i>Carcharhinus amblyrhynchos</i>	<i>Carcharhinus plumbeus</i>	27.52	89.09	4.03E-10	****
6	<i>Carcharhinus amblyrhynchos</i>	<i>Galeocerdo cuvier</i>	4.64	51.74	0.003	**
7	<i>Carcharhinus amblyrhynchos</i>	<i>Loxodon macrorhinus</i>	3.32	61.14	0.114	ns
8	<i>Carcharhinus amblyrhynchos</i>	<i>Rhizoprionodon taylori</i>	7.87	51.64	3.15E-08	****
9	<i>Carcharhinus amblyrhynchos</i>	<i>Rhizoprionodon terraenovae</i>	76.04	68.62	0	****
10	<i>Carcharhinus amblyrhynchos</i>	<i>Scoliodon laticaudus</i>	67.63	57.85	1.44E-11	****
11	<i>Carcharhinus amblyrhynchos</i>	<i>Scyliorhinus canicula</i>	1.59	139.08	0.98	ns

	<i>Carcharhinus</i>					
12	<i>amblyrhynchos</i>	<i>Sphyrna lewini</i>	10.47	123.52	1.75E-13	****
	<i>Carcharhinus</i>					
13	<i>amblyrhynchos</i>	<i>Sphyrna tiburo</i>	16.81	80.77	1.62E-11	****
	<i>Carcharhinus</i>					
14	<i>amblyrhynchos</i>	<i>Sphyrna zygaena</i>	4.54	35.29	0.006	**
	<i>Carcharhinus</i>					
15	<i>amblyrhynchos</i>	<i>Squalus acanthias</i>	8.81	95.29	4.63E-10	****
	<i>Carcharhinus</i>					
16	<i>amblyrhynchos</i>	<i>Squalus megalops</i>	9.40	65.28	1.32E-11	****
	<i>Carcharhinus</i>					
17	<i>amblyrhynchos</i>	<i>Triaenodon obesus</i>	7.03	53.85	5.51E-07	****
18	<i>Carcharhinus brachyurus</i>	<i>Carcharhinus falciformis</i>	6.61	161.75	7.83E-08	****
19	<i>Carcharhinus brachyurus</i>	<i>Carcharhinus limbatus</i>	20.99	51.91	0	****
20	<i>Carcharhinus brachyurus</i>	<i>Carcharhinus perezii</i>	41.07	62.62	1.70E-11	****
21	<i>Carcharhinus brachyurus</i>	<i>Carcharhinus plumbeus</i>	32.85	163.14	0	****
22	<i>Carcharhinus brachyurus</i>	<i>Galeocerdo cuvier</i>	9.27	59.84	7.31E-11	****
23	<i>Carcharhinus brachyurus</i>	<i>Loxodon macrorhinus</i>	5.11	139.65	0.000147	***
24	<i>Carcharhinus brachyurus</i>	<i>Rhizoprionodon taylori</i>	15.66	127.27	0	****
		<i>Rhizoprionodon</i>				
25	<i>Carcharhinus brachyurus</i>	<i>terraenovae</i>	77.96	134.96	2.04E-14	****
26	<i>Carcharhinus brachyurus</i>	<i>Scoliodon laticaudus</i>	69.86	138.61	0	****
27	<i>Carcharhinus brachyurus</i>	<i>Scyliorhinus canicula</i>	4.92	149.86	0.000311	***
28	<i>Carcharhinus brachyurus</i>	<i>Sphyrna lewini</i>	14.87	147.75	0	****
29	<i>Carcharhinus brachyurus</i>	<i>Sphyrna tiburo</i>	19.14	83.48	1.33E-10	****
30	<i>Carcharhinus brachyurus</i>	<i>Sphyrna zygaena</i>	7.53	37.07	7.74E-07	****
31	<i>Carcharhinus brachyurus</i>	<i>Squalus acanthias</i>	15.53	195.67	0	****
32	<i>Carcharhinus brachyurus</i>	<i>Squalus megalops</i>	16.52	137.97	1.50E-14	****
33	<i>Carcharhinus brachyurus</i>	<i>Triaenodon obesus</i>	0.84	69.60	1	ns
34	<i>Carcharhinus falciformis</i>	<i>Carcharhinus limbatus</i>	18.46	53.21	0	****
35	<i>Carcharhinus falciformis</i>	<i>Carcharhinus perezii</i>	34.10	68.72	0	****
36	<i>Carcharhinus falciformis</i>	<i>Carcharhinus plumbeus</i>	24.03	150.28	0	****
37	<i>Carcharhinus falciformis</i>	<i>Galeocerdo cuvier</i>	4.21	64.89	0.009	**
38	<i>Carcharhinus falciformis</i>	<i>Loxodon macrorhinus</i>	3.11	121.90	0.169	ns
39	<i>Carcharhinus falciformis</i>	<i>Rhizoprionodon taylori</i>	6.26	110.26	1.15E-06	****
		<i>Rhizoprionodon</i>				
40	<i>Carcharhinus falciformis</i>	<i>terraenovae</i>	65.54	125.21	2.84E-14	****
41	<i>Carcharhinus falciformis</i>	<i>Scoliodon laticaudus</i>	56.60	119.02	0	****
42	<i>Carcharhinus falciformis</i>	<i>Scyliorhinus canicula</i>	1.42	155.59	0.994	ns
43	<i>Carcharhinus falciformis</i>	<i>Sphyrna lewini</i>	9.74	154.25	1.88E-13	****

44	<i>Carcharhinus falciformis</i>	<i>Sphyrna tiburo</i>	16.47	85.73	2.45E-10	****
45	<i>Carcharhinus falciformis</i>	<i>Sphyrna zygaena</i>	4.33	38.65	0.01	**
46	<i>Carcharhinus falciformis</i>	<i>Squalus acanthias</i>	7.45	166.78	7.43E-10	****
47	<i>Carcharhinus falciformis</i>	<i>Squalus megalops</i>	7.77	124.25	3.83E-10	****
48	<i>Carcharhinus falciformis</i>	<i>Triaenodon obesus</i>	6.67	76.22	5.37E-07	****
49	<i>Carcharhinus limbatus</i>	<i>Carcharhinus perezii</i>	3.61	56.84	0.057	ns
50	<i>Carcharhinus limbatus</i>	<i>Carcharhinus plumbeus</i>	10.34	50.62	2.35E-12	****
51	<i>Carcharhinus limbatus</i>	<i>Galeocerdo cuvier</i>	15.59	62.04	1.88E-11	****
52	<i>Carcharhinus limbatus</i>	<i>Loxodon macrorhinus</i>	19.92	48.09	0	****
53	<i>Carcharhinus limbatus</i>	<i>Rhizoprionodon taylori</i>	17.13	47.38	0	****
		<i>Rhizoprionodon</i>				
54	<i>Carcharhinus limbatus</i>	<i>terraenovae</i>	2.75	49.20	0.375	ns
55	<i>Carcharhinus limbatus</i>	<i>Scoliodon laticaudus</i>	1.57	47.76	0.979	ns
56	<i>Carcharhinus limbatus</i>	<i>Scyliorhinus canicula</i>	15.08	89.18	4.06E-10	****
57	<i>Carcharhinus limbatus</i>	<i>Sphyrna lewini</i>	12.86	64.60	4.91E-12	****
58	<i>Carcharhinus limbatus</i>	<i>Sphyrna tiburo</i>	2.19	109.44	0.757	ns
59	<i>Carcharhinus limbatus</i>	<i>Sphyrna zygaena</i>	12.60	75.56	0	****
60	<i>Carcharhinus limbatus</i>	<i>Squalus acanthias</i>	16.23	50.02	0	****
61	<i>Carcharhinus limbatus</i>	<i>Squalus megalops</i>	16.40	48.64	0	****
62	<i>Carcharhinus limbatus</i>	<i>Triaenodon obesus</i>	20.94	54.97	3.55E-12	****
63	<i>Carcharhinus perezii</i>	<i>Carcharhinus plumbeus</i>	16.20	55.30	4.68E-12	****
64	<i>Carcharhinus perezii</i>	<i>Galeocerdo cuvier</i>	23.55	63.99	9.57E-12	****
65	<i>Carcharhinus perezii</i>	<i>Loxodon macrorhinus</i>	42.50	41.37	9.32E-13	****
66	<i>Carcharhinus perezii</i>	<i>Rhizoprionodon taylori</i>	35.94	37.48	0	****
		<i>Rhizoprionodon</i>				
67	<i>Carcharhinus perezii</i>	<i>terraenovae</i>	16.58	46.85	0	****
68	<i>Carcharhinus perezii</i>	<i>Scoliodon laticaudus</i>	5.90	39.65	8.60E-05	****
69	<i>Carcharhinus perezii</i>	<i>Scyliorhinus canicula</i>	18.95	147.76	1.68E-14	****
70	<i>Carcharhinus perezii</i>	<i>Sphyrna lewini</i>	18.07	110.95	0	****
71	<i>Carcharhinus perezii</i>	<i>Sphyrna tiburo</i>	0.82	90.66	1	ns
72	<i>Carcharhinus perezii</i>	<i>Sphyrna zygaena</i>	14.42	42.83	1.13E-12	****
73	<i>Carcharhinus perezii</i>	<i>Squalus acanthias</i>	31.12	53.14	0	****
74	<i>Carcharhinus perezii</i>	<i>Squalus megalops</i>	32.69	44.09	7.69E-13	****
75	<i>Carcharhinus perezii</i>	<i>Triaenodon obesus</i>	38.14	59.39	1.89E-11	****
76	<i>Carcharhinus plumbeus</i>	<i>Galeocerdo cuvier</i>	13.13	54.27	1.23E-12	****
77	<i>Carcharhinus plumbeus</i>	<i>Loxodon macrorhinus</i>	35.51	137.96	0	****
78	<i>Carcharhinus plumbeus</i>	<i>Rhizoprionodon taylori</i>	26.23	126.10	0	****
		<i>Rhizoprionodon</i>				
79	<i>Carcharhinus plumbeus</i>	<i>terraenovae</i>	46.05	126.64	6.13E-14	****
80	<i>Carcharhinus plumbeus</i>	<i>Scoliodon laticaudus</i>	34.36	139.22	0	****

81	<i>Carcharhinus plumbeus</i>	<i>Scyliorhinus canicula</i>	10.72	143.14	1.89E-13	****
82	<i>Carcharhinus plumbeus</i>	<i>Sphyrna lewini</i>	7.00	137.08	1.57E-08	****
83	<i>Carcharhinus plumbeus</i>	<i>Sphyrna tiburo</i>	7.85	81.20	2.27E-09	****
84	<i>Carcharhinus plumbeus</i>	<i>Sphyrna zygaena</i>	6.59	35.50	1.62E-05	****
85	<i>Carcharhinus plumbeus</i>	<i>Squalus acanthias</i>	19.64	200.83	7.77E-14	****
86	<i>Carcharhinus plumbeus</i>	<i>Squalus megalops</i>	21.57	132.49	1.04E-14	****
87	<i>Carcharhinus plumbeus</i>	<i>Triaenodon obesus</i>	29.26	60.87	2.04E-11	****
88	<i>Galeocerdo cuvier</i>	<i>Loxodon macrorhinus</i>	6.86	43.66	2.63E-06	****
89	<i>Galeocerdo cuvier</i>	<i>Rhizoprionodon taylori</i>	0.59	40.71	1	ns
		<i>Rhizoprionodon</i>				
90	<i>Galeocerdo cuvier</i>	<i>terraenovae</i>	41.93	47.99	0	****
91	<i>Galeocerdo cuvier</i>	<i>Scoliodon laticaudus</i>	33.87	42.33	1.12E-12	****
92	<i>Galeocerdo cuvier</i>	<i>Scyliorhinus canicula</i>	1.43	149.08	0.994	ns
93	<i>Galeocerdo cuvier</i>	<i>Sphyrna lewini</i>	4.73	104.00	0.000955	***
94	<i>Galeocerdo cuvier</i>	<i>Sphyrna tiburo</i>	13.50	98.08	3.02E-10	****
95	<i>Galeocerdo cuvier</i>	<i>Sphyrna zygaena</i>	1.48	48.93	0.988	ns
96	<i>Galeocerdo cuvier</i>	<i>Squalus acanthias</i>	0.87	52.30	1	ns
97	<i>Galeocerdo cuvier</i>	<i>Squalus megalops</i>	0.78	45.80	1	ns
98	<i>Galeocerdo cuvier</i>	<i>Triaenodon obesus</i>	9.27	61.98	5.81E-11	****
99	<i>Loxodon macrorhinus</i>	<i>Rhizoprionodon taylori</i>	14.89	184.04	0	****
		<i>Rhizoprionodon</i>				
100	<i>Loxodon macrorhinus</i>	<i>terraenovae</i>	95.71	106.50	2.16E-14	****
101	<i>Loxodon macrorhinus</i>	<i>Scoliodon laticaudus</i>	90.98	205.06	0	****
102	<i>Loxodon macrorhinus</i>	<i>Scyliorhinus canicula</i>	2.94	129.82	0.246	ns
103	<i>Loxodon macrorhinus</i>	<i>Sphyrna lewini</i>	13.00	115.18	3.60E-14	****
104	<i>Loxodon macrorhinus</i>	<i>Sphyrna tiburo</i>	17.99	76.74	0	****
105	<i>Loxodon macrorhinus</i>	<i>Sphyrna zygaena</i>	5.82	32.47	0.00021	***
106	<i>Loxodon macrorhinus</i>	<i>Squalus acanthias</i>	13.92	247.79	1.51E-13	****
107	<i>Loxodon macrorhinus</i>	<i>Squalus megalops</i>	15.52	125.84	0	****
108	<i>Loxodon macrorhinus</i>	<i>Triaenodon obesus</i>	5.22	44.02	0.000583	***
		<i>Rhizoprionodon</i>				
109	<i>Rhizoprionodon taylori</i>	<i>terraenovae</i>	89.96	94.49	4.76E-10	****
110	<i>Rhizoprionodon taylori</i>	<i>Scoliodon laticaudus</i>	85.18	318.68	9.39E-13	****
111	<i>Rhizoprionodon taylori</i>	<i>Scyliorhinus canicula</i>	1.29	125.92	0.998	ns
112	<i>Rhizoprionodon taylori</i>	<i>Sphyrna lewini</i>	6.95	108.44	4.22E-08	****
113	<i>Rhizoprionodon taylori</i>	<i>Sphyrna tiburo</i>	15.03	75.47	0	****
114	<i>Rhizoprionodon taylori</i>	<i>Sphyrna zygaena</i>	2.04	31.62	0.828	ns
115	<i>Rhizoprionodon taylori</i>	<i>Squalus acanthias</i>	2.84	267.06	0.294	ns
116	<i>Rhizoprionodon taylori</i>	<i>Squalus megalops</i>	3.02	118.08	0.208	ns
117	<i>Rhizoprionodon taylori</i>	<i>Triaenodon obesus</i>	13.83	39.25	2.07E-13	****

118	<i>Rhizoprionodon terraenovae</i>	<i>Scoliodon laticaudus</i>	18.90	107.41	1.69E-14	****
119	<i>Rhizoprionodon terraenovae</i>	<i>Scyliorhinus canicula</i>	30.21	135.19	0	****
120	<i>Rhizoprionodon terraenovae</i>	<i>Sphyrna lewini</i>	35.08	122.91	2.53E-14	****
121	<i>Rhizoprionodon terraenovae</i>	<i>Sphyrna tiburo</i>	6.05	78.68	6.66E-06	****
122	<i>Rhizoprionodon terraenovae</i>	<i>Sphyrna zygaena</i>	24.09	33.80	0	****
123	<i>Rhizoprionodon terraenovae</i>	<i>Squalus acanthias</i>	69.41	168.90	6.11E-15	****
124	<i>Rhizoprionodon terraenovae</i>	<i>Squalus megalops</i>	77.13	102.22	4.37E-14	****
125	<i>Rhizoprionodon terraenovae</i>	<i>Triaenodon obesus</i>	67.03	50.41	0	****
126	<i>Scoliodon laticaudus</i>	<i>Scyliorhinus canicula</i>	24.29	128.10	1.42E-14	****
127	<i>Scoliodon laticaudus</i>	<i>Sphyrna lewini</i>	26.90	112.46	5.12E-14	****
128	<i>Scoliodon laticaudus</i>	<i>Sphyrna tiburo</i>	1.49	76.15	0.989	ns
129	<i>Scoliodon laticaudus</i>	<i>Sphyrna zygaena</i>	18.64	32.08	1.01E-13	****
130	<i>Scoliodon laticaudus</i>	<i>Squalus acanthias</i>	60.18	287.90	5.47E-13	****
131	<i>Scoliodon laticaudus</i>	<i>Squalus megalops</i>	68.96	134.65	0	****
132	<i>Scoliodon laticaudus</i>	<i>Triaenodon obesus</i>	58.68	41.95	1.05E-12	****
133	<i>Scyliorhinus canicula</i>	<i>Sphyrna lewini</i>	5.08	196.91	0.000125	***
134	<i>Scyliorhinus canicula</i>	<i>Sphyrna tiburo</i>	13.12	142.60	9.10E-15	****
135	<i>Scyliorhinus canicula</i>	<i>Sphyrna zygaena</i>	2.41	83.65	0.6	ns
136	<i>Scyliorhinus canicula</i>	<i>Squalus acanthias</i>	2.23	141.00	0.731	ns
137	<i>Scyliorhinus canicula</i>	<i>Squalus megalops</i>	2.18	132.51	0.76	ns
138	<i>Scyliorhinus canicula</i>	<i>Triaenodon obesus</i>	5.21	147.75	9.04E-05	****
139	<i>Sphyrna lewini</i>	<i>Sphyrna tiburo</i>	10.64	104.95	8.60E-14	****
140	<i>Sphyrna lewini</i>	<i>Sphyrna zygaena</i>	1.91	52.81	0.897	ns
141	<i>Sphyrna lewini</i>	<i>Squalus acanthias</i>	5.13	136.09	0.000137	***
142	<i>Sphyrna lewini</i>	<i>Squalus megalops</i>	5.41	119.17	4.76E-05	****
143	<i>Sphyrna lewini</i>	<i>Triaenodon obesus</i>	14.51	117.61	1.55E-15	****
144	<i>Sphyrna tiburo</i>	<i>Sphyrna zygaena</i>	10.59	98.73	2.48E-10	****
145	<i>Sphyrna tiburo</i>	<i>Squalus acanthias</i>	14.09	80.20	0	****
146	<i>Sphyrna tiburo</i>	<i>Squalus megalops</i>	14.25	77.69	0	****
147	<i>Sphyrna tiburo</i>	<i>Triaenodon obesus</i>	19.11	87.94	3.53E-10	****
148	<i>Sphyrna zygaena</i>	<i>Squalus acanthias</i>	1.11	34.79	0.999	ns
149	<i>Sphyrna zygaena</i>	<i>Squalus megalops</i>	1.19	33.12	0.999	ns
150	<i>Sphyrna zygaena</i>	<i>Triaenodon obesus</i>	7.73	40.64	2.38E-07	****
151	<i>Squalus acanthias</i>	<i>Squalus megalops</i>	0.20	199.20	1	ns
152	<i>Squalus acanthias</i>	<i>Triaenodon obesus</i>	14.21	58.83	1.75E-11	****
153	<i>Squalus megalops</i>	<i>Triaenodon obesus</i>	14.82	47.23	0	****

Table 4: Games-Howell pairwise comparison test of $\delta^{15}\text{Nc}$ (‰) between location groups.

Test #	Group1	Group2	Statistic	df	p-value	Significance
1	Aomori	Balearic Island	14.44	99.31	1.94E-10	****
2	Aomori	CE Pacific	7.76	98.39	1.52E-09	****
3	Aomori	English Channel	7.69	66.34	1.39E-08	****
4	Aomori	Florida	7.46	118.17	2.46E-09	****
5	Aomori	Galveston	14.06	206.44	0	****
6	Aomori	Great Barrier Reef	3.54	126.60	0.053	ns
7	Aomori	Gulf St. Vincent	4.47	44.36	0.006	**
8	Aomori	Hong Kong	34.31	87.14	3.16E-10	****
9	Aomori	KZN Nets	12.59	89.99	4.35E-10	****
10	Aomori	Mayotte	4.57	102.94	0.002	**
11	Aomori	New Providence	24.70	101.18	2.86E-14	****
12	Aomori	North Sea	6.91	146.03	2.14E-08	****
13	Aomori	Nosy Hao	2.29	99.98	0.686	ns
14	Aomori	Queensland	2.33	86.56	0.657	ns
15	Aomori	Reunion Island	3.34	92.59	0.099	ns
16	Aomori	Spencer Gulf	0.46	76.93	1	ns
17	Aomori	St Helena	14.87	106.36	0	****
18	Balearic Island	CE Pacific	15.44	75.43	0	****
19	Balearic Island	English Channel	18.87	40.76	7.51E-13	****
20	Balearic Island	Florida	24.70	66.75	0	****
21	Balearic Island	Galveston	29.46	164.87	4.66E-15	****
22	Balearic Island	Great Barrier Reef	14.18	103.81	4.17E-14	****
23	Balearic Island	Gulf St. Vincent	11.45	34.23	4.27E-11	****
24	Balearic Island	Hong Kong	98.52	106.61	3.77E-14	****
25	Balearic Island	KZN Nets	52.96	96.84	3.86E-10	****
26	Balearic Island	Mayotte	13.28	54.88	3.20E-12	****
27	Balearic Island	New Providence	53.62	49.71	0	****
28	Balearic Island	North Sea	28.92	171.64	0	****
29	Balearic Island	Nosy Hao	22.10	143.69	2.03E-14	****
30	Balearic Island	Queensland	34.99	105.09	1.78E-15	****
31	Balearic Island	Reunion Island	34.21	104.79	9.88E-15	****
32	Balearic Island	Spencer Gulf	6.83	57.47	8.47E-07	****
33	Balearic Island	St Helena	50.03	129.92	2.71E-14	****
34	CE Pacific	English Channel	1.77	104.96	0.948	ns
35	CE Pacific	Florida	3.46	94.71	0.071	ns

36	CE Pacific	Galveston	1.37	113.82	0.996	ns
37	CE Pacific	Great Barrier Reef	10.02	83.43	1.31E-10	****
38	CE Pacific	Gulf St. Vincent	2.31	83.79	0.675	ns
39	CE Pacific	Hong Kong	7.79	72.57	5.38E-09	****
40	CE Pacific	KZN Nets	2.41	73.33	0.6	ns
41	CE Pacific	Mayotte	10.58	81.22	3.35E-11	****
42	CE Pacific	New Providence	5.35	84.98	0.000103	***
43	CE Pacific	North Sea	4.40	85.39	0.004	**
44	CE Pacific	Nosy Hao	9.50	75.12	0	****
45	CE Pacific	Queensland	7.36	72.45	3.51E-08	****
46	CE Pacific	Reunion Island	6.81	73.84	3.28E-07	****
47	CE Pacific	Spencer Gulf	6.50	121.76	2.73E-07	****
48	CE Pacific	St Helena	0.96	76.71	1	ns
49	English					
	Channel	Florida	2.00	61.79	0.86	ns
50	English					
	Channel	Galveston	4.18	84.87	0.008	**
51	English					
	Channel	Great Barrier Reef	10.99	49.53	0	****
52	English					
	Channel	Gulf St. Vincent	0.96	55.79	1	ns
53	English					
	Channel	Hong Kong	14.18	37.71	0	****
54	English					
	Channel	KZN Nets	0.41	38.51	1	ns
55	English					
	Channel	Mayotte	11.79	46.97	0	****
56	English					
	Channel	New Providence	10.08	51.00	1.04E-11	****
57	English					
	Channel	North Sea	3.24	51.81	0.142	ns
58	English					
	Channel	Nosy Hao	10.47	40.43	6.57E-11	****
59	English					
	Channel	Queensland	7.48	37.58	8.37E-07	****
60	English					
	Channel	Reunion Island	6.68	39.06	7.97E-06	****
61	English					
	Channel	Spencer Gulf	5.80	86.84	1.51E-05	****

	English					
62	Channel	St Helena	1.61	42.15	0.973	ns
63	Florida	Galveston	7.56	168.88	3.78E-10	****
64	Florida	Great Barrier Reef	12.41	90.72	4.57E-10	****
65	Florida	Gulf St. Vincent	0.37	43.16	1	ns
66	Florida	Hong Kong	25.22	57.27	1.23E-11	****
67	Florida	KZN Nets	2.88	59.60	0.297	ns
68	Florida	Mayotte	13.63	75.50	0	****
69	Florida	New Providence	16.83	78.00	0	****
70	Florida	North Sea	1.54	103.36	0.985	ns
71	Florida	Nosy Hao	12.24	66.63	0	****
72	Florida	Queensland	7.88	56.85	1.63E-08	****
73	Florida	Reunion Island	6.61	61.48	1.52E-06	****
74	Florida	Spencer Gulf	4.95	74.04	0.000601	***
75	Florida	St Helena	5.70	71.82	3.55E-05	****
76	Galveston	Great Barrier Reef	18.99	192.82	0	****
77	Galveston	Gulf St. Vincent	4.12	50.81	0.015	*
78	Galveston	Hong Kong	11.07	151.18	6.51E-14	****
79	Galveston	KZN Nets	6.97	154.26	1.31E-08	****
80	Galveston	Mayotte	20.09	161.79	0	****
81	Galveston	New Providence	6.29	154.51	4.80E-07	****
82	Galveston	North Sea	9.63	216.96	1.51E-13	****
83	Galveston	Nosy Hao	19.21	166.38	0	****
84	Galveston	Queensland	15.80	150.49	1.18E-14	****
85	Galveston	Reunion Island	14.67	157.37	3.77E-15	****
86	Galveston	Spencer Gulf	9.54	89.91	4.33E-10	****
87	Galveston	St Helena	4.27	173.27	0.004	**
	Great Barrier Reef					
88		Gulf St. Vincent	6.44	37.63	1.95E-05	****
	Great Barrier Reef					
89		Hong Kong	53.95	87.65	3.40E-10	****
	Great Barrier Reef					
90		KZN Nets	23.12	90.43	4.49E-10	****
	Great Barrier Reef					
91		Mayotte	1.23	82.67	0.999	ns
	Great Barrier Reef					
92		New Providence	34.63	74.92	0	****
	Great Barrier Reef					
93		North Sea	12.92	170.33	0	****

	Great Barrier					
94	Reef	Nosy Hao	2.26	110.75	0.71	ns
	Great Barrier					
95	Reef	Queensland	9.08	86.63	2.96E-10	****
	Great Barrier					
96	Reef	Reunion Island	10.16	95.11	4.61E-10	****
	Great Barrier					
97	Reef	Spencer Gulf	1.45	64.15	0.991	ns
	Great Barrier					
98	Reef	St Helena	24.96	117.26	2.25E-14	****
99	Gulf St. Vincent	Hong Kong	10.49	33.04	6.84E-10	****
100	Gulf St. Vincent	KZN Nets	0.86	33.36	1	ns
101	Gulf St. Vincent	Mayotte	6.95	36.88	4.57E-06	****
102	Gulf St. Vincent	New Providence	8.08	38.72	1.10E-07	****
103	Gulf St. Vincent	North Sea	1.14	38.35	0.999	ns
104	Gulf St. Vincent	Nosy Hao	5.84	34.08	0.000168	***
105	Gulf St. Vincent	Queensland	3.79	33.00	0.048	*
106	Gulf St. Vincent	Reunion Island	3.29	33.57	0.145	ns
107	Gulf St. Vincent	Spencer Gulf	3.95	75.43	0.018	*
108	Gulf St. Vincent	St Helena	2.20	34.75	0.74	ns
109	Hong Kong	KZN Nets	50.13	125.22	0	****
110	Hong Kong	Mayotte	58.22	42.15	1.08E-12	****
111	Hong Kong	New Providence	5.90	39.65	8.61E-05	****
112	Hong Kong	North Sea	35.13	160.58	0	****
113	Hong Kong	Nosy Hao	75.06	233.66	0	****
114	Hong Kong	Queensland	80.87	354.65	0	****
115	Hong Kong	Reunion Island	68.96	134.65	0	****
116	Hong Kong	Spencer Gulf	17.38	55.11	3.99E-12	****
117	Hong Kong	St Helena	34.36	139.22	2.23E-14	****
118	KZN Nets	Mayotte	25.78	45.07	1.84E-13	****
119	KZN Nets	New Providence	21.34	42.07	1.08E-12	****
120	KZN Nets	North Sea	6.03	159.08	1.62E-06	****
121	KZN Nets	Nosy Hao	29.46	158.38	9.26E-14	****
122	KZN Nets	Queensland	24.47	124.40	0	****
123	KZN Nets	Reunion Island	19.67	102.97	3.35E-14	****
124	KZN Nets	Spencer Gulf	6.76	55.73	1.29E-06	****
125	KZN Nets	St Helena	5.45	124.03	3.74E-05	****
126	Mayotte	New Providence	36.66	57.80	1.42E-11	****
127	Mayotte	North Sea	14.46	106.36	0	****

128	Mayotte	Nosy Hao	3.86	55.96	0.029	*
129	Mayotte	Queensland	11.16	41.54	7.65E-12	****
130	Mayotte	Reunion Island	12.18	47.82	0	****
131	Mayotte	Spencer Gulf	1.99	62.41	0.864	ns
132	Mayotte	St Helena	27.35	62.70	1.67E-11	****
	New					
133	Providence	North Sea	21.54	90.09	4.38E-10	****
	New					
134	Providence	Nosy Hao	38.76	49.73	0	****
	New					
135	Providence	Queensland	35.26	39.20	1.99E-13	****
	New					
136	Providence	Reunion Island	32.69	44.09	7.69E-13	****
	New					
137	Providence	Spencer Gulf	14.36	65.70	0	****
	New					
138	Providence	St Helena	16.20	55.30	4.68E-12	****
139	North Sea	Nosy Hao	13.24	192.78	0	****
140	North Sea	Queensland	7.85	159.19	8.66E-11	****
141	North Sea	Reunion Island	6.16	165.85	8.07E-07	****
142	North Sea	Spencer Gulf	4.32	65.72	0.006	**
143	North Sea	St Helena	9.25	191.89	1.21E-13	****
144	Nosy Hao	Queensland	9.72	235.27	1.61E-13	****
145	Nosy Hao	Reunion Island	10.95	167.95	1.24E-13	****
146	Nosy Hao	Spencer Gulf	0.66	57.20	1	ns
147	Nosy Hao	St Helena	29.95	176.63	0	****
148	Queensland	Reunion Island	2.42	133.79	0.591	ns
149	Queensland	Spencer Gulf	1.65	55.01	0.967	ns
150	Queensland	St Helena	25.19	137.76	3.02E-14	****
151	Reunion Island	Spencer Gulf	2.17	56.16	0.763	ns
152	Reunion Island	St Helena	21.57	132.49	1.04E-14	****
153	Spencer Gulf	St Helena	8.15	58.52	4.80E-09	****

Table 5: Linear regression model outputs assessing the relationship between $\delta^{15}\text{Nc}$ (‰) and total length (TLcm).

Species	Intercept (d15Nc)	Estimate (TLcm)	Residual SE	Adjusted R ²	F- statistic	p-value
<i>Carcharhinus</i>						
<i>amblyrhynchos</i>	7.71	0.00	0.53	0.03	1.95	0.173
<i>Carcharhinus brachyurus</i>	8.21	-0.01	0.99	0.05	5.72	0.019
<i>Carcharhinus falciformis</i>	8.93	-0.01	0.98	0.04	3.77	0.056
<i>Carcharhinus limbatus</i>	25.43	-0.06	2.24	0.43	35.32	<0.001
<i>Carcharhinus perezii</i>	14.05	0.01	0.84	0.02	1.50	0.231
<i>Carcharhinus plumbeus</i>	12.18	0.00	0.86	-0.01	0.54	0.464
<i>Galeocerdo cuvier</i>	8.09	0.01	1.12	0.00	1.02	0.321
<i>Loxodon macrorhinus</i>	7.85	0.00	0.59	-0.01	0.00	0.948
<i>Rhizoprionodon taylori</i>	7.48	0.02	0.65	0.05	9.17	0.003
<i>Rhizoprionodon terraenovae</i>	17.75	0.00	0.57	-0.02	0.01	0.934
<i>Scoliodon laticaudus</i>	16.05	-0.01	0.76	0.01	2.05	0.155
<i>Scyliorhinus canicula</i>	4.71	0.09	2.92	0.13	19.39	<0.001
<i>Sphyrna lewini</i>	11.76	-0.01	1.78	0.09	9.97	0.002
<i>Sphyrna tiburo</i>	6.47	0.11	1.57	0.76	210.50	<0.001
<i>Sphyrna zygaena</i>	12.57	-0.02	1.77	0.03	1.84	0.185
<i>Squalus acanthias</i>	7.48	0.02	1.11	0.08	15.82	<0.001
<i>Squalus megalops</i>	7.55	0.03	0.36	0.56	72.96	<0.001
<i>Triaenodon obesus</i>	4.99	0.02	0.71	0.13	5.59	0.025

Figure 1: QQ plots for a) M9 and b) M13.

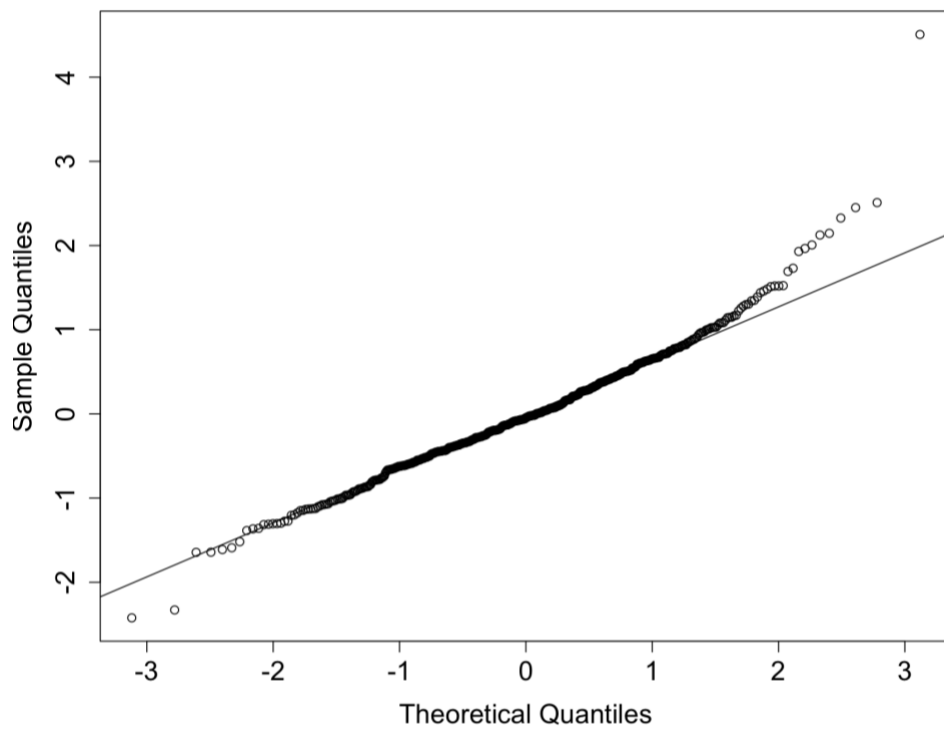
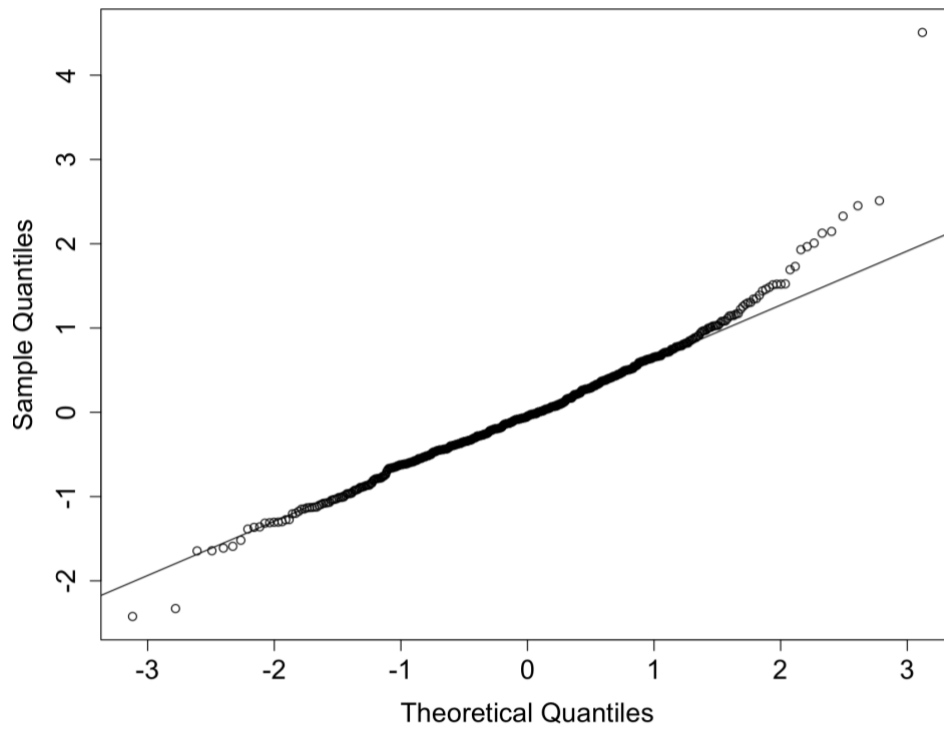


Figure 2: Histograms of residuals for a) M9 and b) M13.

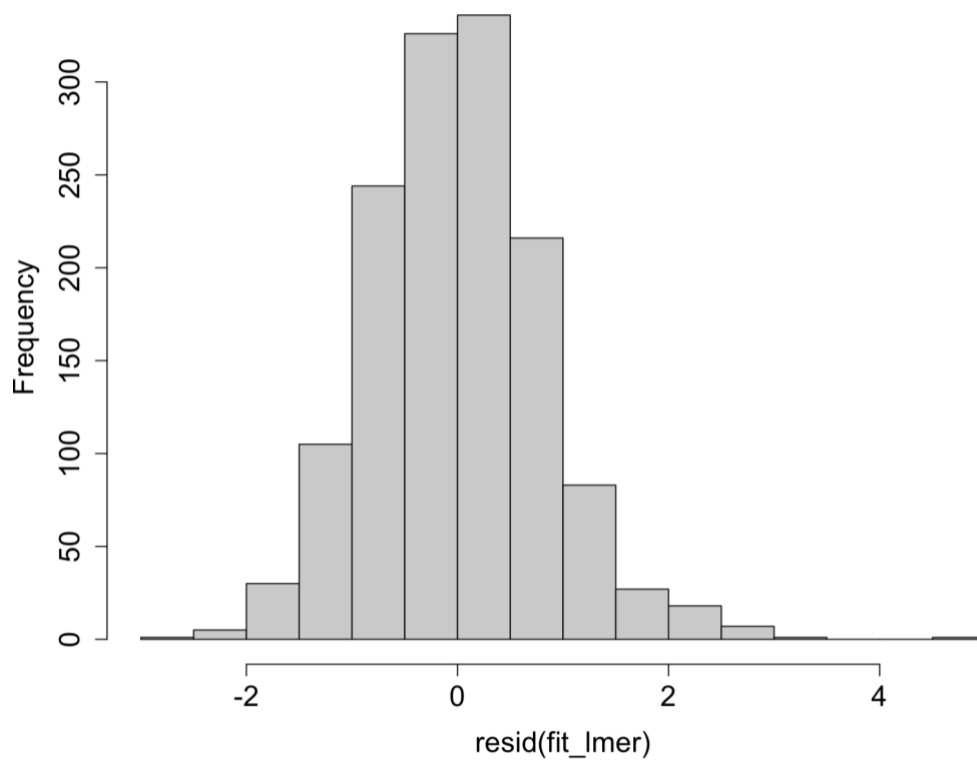
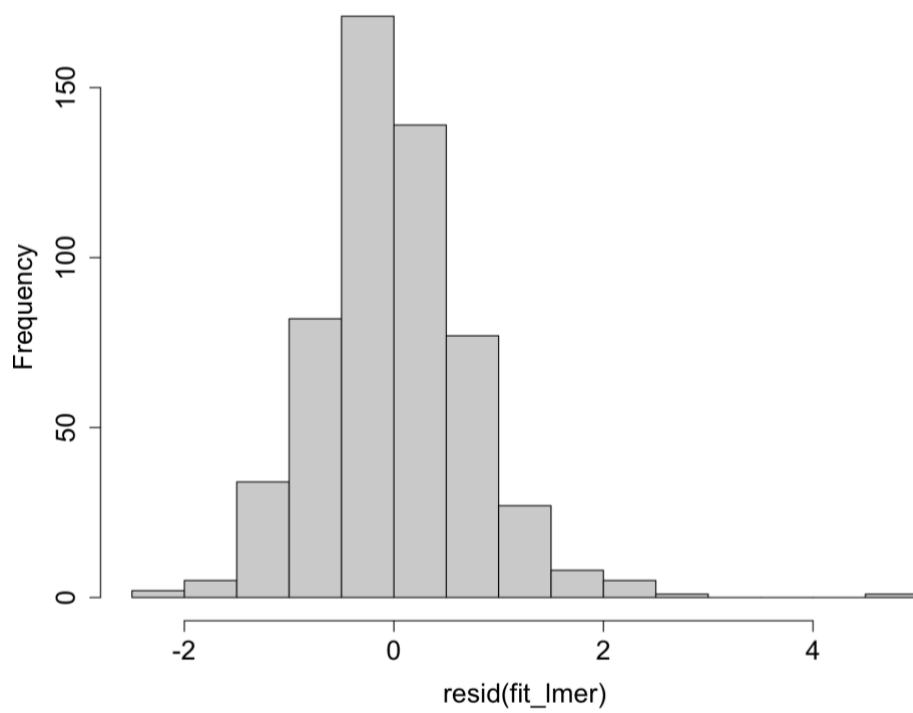


Figure 3: Residual plots of a) M9 and b) M13.

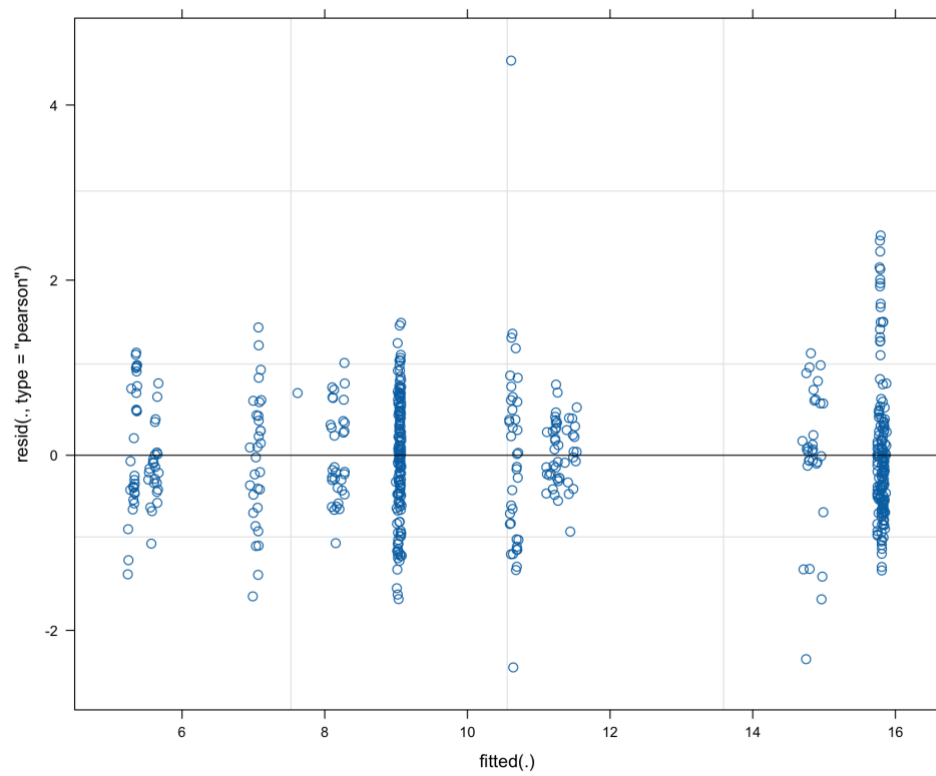
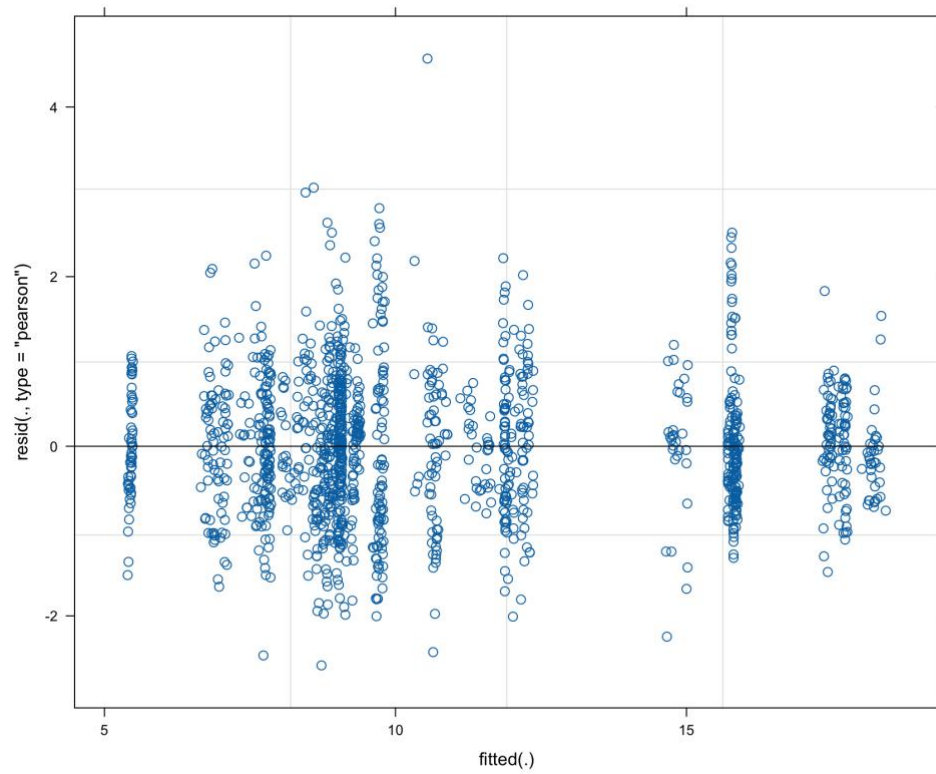


Table 5: Eigenvalues and proportion of variance explained applying principal component analysis to $\delta^{15}\text{Nc}$ (‰), total length (TLcm), and depth data (standardised).

	PC1	PC2	PC3
Eigenvalue	1.15	1.06	0.75
Proportion of variance	0.44	0.38	0.19
Cumulative proportion	0.44	0.81	1.00

Table 6: Principal component analysis correlations for $\delta^{15}\text{Nc}$ (‰), total length (TLcm), and depth data (standardised).

	PC1	PC2
Depth	0.848	-0.237
Total length (TLcm)	-0.749	-0.484
$\delta^{15}\text{Nc}$ (‰)	-0.177	0.915

Appendix III

Table 1: Beach litter survey sites (N = 40) detailing categorical variables and average macrolitter items/m².

Site #	Survey site	Categorical variables						Transect approach (items per m ²)			
		Location	Beach type	Sediment	Exposure	Cleanup	Existing policy	Parallel	SD	Perpendicular	SD
B33	Balta - Rocky Beach 1	Offshore	Remote	Pebble	Sheltered	No	Not known	0.16	-	0.54	0.55
B31	Balta - Sandy Beach 1	Offshore	Remote	Sand	Sheltered	No	Not known	0.11	-	0.07	0.05
B32	Balta - Sandy Beach 2	Offshore	Remote	Sand	Sheltered	No	Not known	0.03	-	0.03	0.03
B14	Black Edge, Samson	Offshore	Remote	Sand	Sheltered	Yes	Yes	0.03	-	0.02	0.03
B3	Broadsands Beach	Mainland	Urban	Sand	Sheltered	Yes	Yes	0.01	-	0.09	0.11
B4	Cawsands Beach	Mainland	Semi-urban	Pebble	Sheltered	Yes	Yes	0.09	-	0.13	0.13
B38	Clinkimins reach (Shetland)	Offshore	Semi-urban	Sand	Sheltered	No	Not known	0.05	-	0.14	0.08
B2	Elberry Cove	Mainland	Urban	Pebble	Sheltered	Yes	Yes	1.96	-	0.44	0.35
B34	Grutness (Shetland)	Offshore	Remote	Sand	Sheltered	Yes	Yes	0.11	-	0.05	0.02
B8	Helford passage	Mainland	Rural	Pebble	Sheltered	Yes	Yes	0.07	-	0.09	0.10
B19	Holyhead	Offshore	Remote	Mixed	Sheltered	Yes	Not known	0.08	-	0.08	0.05
B20	Isle of Aaron	Offshore	Semi-urban	Sand	Sheltered	Yes	Yes	0.09	-	0.05	0.04
B5	Kingsands	Mainland	Semi-urban	Pebble	Sheltered	Yes	Yes	0.22	-	0.10	0.09
B13	Landing beach (Scilly)	Offshore	Remote	Sand	Sheltered	Yes	Yes	0.03	-	0.02	0.00
B6	Long Rock	Mainland	Urban	Sand	Sheltered	Yes	Yes	0.18	0.14	0.03	0.02
B16	Lundy	Offshore	Remote	Pebble	Sheltered	Yes	Yes	0.11	-	0.06	0.03
B21	Luskentyre (Hebrides)	Offshore	Remote	Sand	Sheltered	No	Not known	0.01	0.00	0.00	0.00
B28	Ness Point (Orkney)	Offshore	Remote	Pebble	Sheltered	Yes	Yes	0.10	-	0.12	0.05
B27	Plockton	Mainland	Rural	Pebble	Sheltered	No	Not known	1.25	-	1.20	0.39
B7	Polgwidden cove	Mainland	Remote	Pebble	Sheltered	Yes	Yes	0.03	-	0.03	0.01
B18	Porth Tywyn-mawr	Offshore	Remote	Sand	Sheltered	Yes	Not known	0.14	-	0.09	0.04
B15	Porthcressa (Scilly)	Offshore	Urban	Sand	Sheltered	Yes	Yes	0.40	-	0.23	0.10
B40	Portobello West	Mainland	Urban	Sand	Sheltered	Yes	Not known	0.17	-	0.48	0.23
B30	Rackwick (Orkney)	Offshore	Remote	Sand	Exposed	Yes	Yes	0.24	-	0.18	0.19
B1	Sandy beach	Mainland	Semi-urban	Sand	Exposed	Yes	Yes	0.02	0.02	0.10	0.05
B39	Seafield	Mainland	Semi-urban	Mixed	Sheltered	Yes	Not known	0.23	-	0.15	0.01
B36	Spiggie (Shetlands)	Offshore	Remote	Sand	Sheltered	Yes	Not known	0.13	-	0.20	0.07
B9	St Agnes 1 (Scilly)	Offshore	Remote	Sand	Sheltered	Yes	Yes	0.07	-	0.01	0.02
B10	St Agnes 2 (Scilly)	Offshore	Remote	Sand	Sheltered	Yes	Yes	0.05	-	0.09	0.08
B37	St Ninians (Shetlands)	Offshore	Remote	Sand	Sheltered	Yes	Yes	0.12	-	0.10	0.03
B24	Stein Inn (Skye)	Offshore	Rural	Pebble	Sheltered	No	Yes	1.46	-	1.72	2.00
B22	Traigh a Bhaigh (Hebrides)	Offshore	Remote	Mixed	Sheltered	No	Not known	0.39	0.48	0.09	0.07
B23	Traigh Siar (Hebrides)	Offshore	Remote	Sand	Sheltered	No	Not known	0.02	0.02	0.03	0.01
B17	Trearddur bay	Offshore	Urban	Sand	Sheltered	Yes	Yes	0.18	-	0.04	0.02
B11	Tresco (Scilly)	Offshore	Remote	Sand	Exposed	Yes	Yes	0.10	-	0.03	0.00
B25	Ullapool	Mainland	Semi-urban	Pebble	Sheltered	No	Yes	0.35	0.11	0.69	0.49
B29	Unknown beach (Orkney)	Offshore	Rural	Pebble	Sheltered	Yes	Yes	1.70	-	0.26	0.10
B26	Upper Loch Torridon	Mainland	Remote	Pebble	Sheltered	No	Not known	0.27	-	0.02	0.02
B12	West Porth (Scilly)	Offshore	Remote	Pebble	Sheltered	Yes	Yes	0.04	-	0.03	0.04
B35	West Voe (Shetlands)	Offshore	Remote	Sand	Sheltered	Yes	Yes	0.10	-	0.11	0.03

Table 2: Average composition of macrolitter (items/m²) and standard deviation) for parallel and perpendicular transect approaches.

Composition of macrolitter	Items m ²			
	Parallel		Perpendicular	
	Mean	SD	Mean	SD
Macroplastic	0.067	0.168	0.040	0.044
Hygiene	0.003	0.007	0.002	0.007
Fishing and rope	0.096	0.218	0.086	0.225
Rubber	0.003	0.008	0.002	0.005
Metal	0.011	0.025	0.012	0.023
Wood	0.004	0.009	0.001	0.002
Paper	0.000	0.002	0.001	0.002
Textile	0.004	0.017	0.002	0.005
Ceramic	0.035	0.133	0.016	0.058
Glass	0.045	0.139	0.036	0.103
Other	0.002	0.005	0.001	0.002

Table 3: Pairwise t-tests on the effect of transect approach on average abundance (items/m²) across beach sites (N = 40) for parallel and perpendicular transect approaches.

Material Category	Test	P Value
Ceramic	Student's t-test	0.45
Fishing and rope	Student's t-test	0.88
Glass	Student's t-test	0.76
Hygiene	Student's t-test	0.46
Macroplastic	Student's t-test	0.37
Metal	Student's t-test	0.92
Other	Student's t-test	0.21
Paper	Student's t-test	0.72
Rubber	Student's t-test	0.43
Textile	Student's t-test	0.54
Wood	Student's t-test	0.07
Mesoplastic	Student's t-test	0.73

Table 4: Principal Coordinate Analysis (PCoA) coefficients for the first two principal components of litter material category abundance data from parallel and perpendicular transects.

Material	Parallel		Perpendicular	
	PC1	PC2	PC1	PC2
Macroplastic	-0.52	-1.32	-0.05	0.77
Hygiene	-0.25	0.55	0.08	0.1
Fishing and rope	-0.95	0.84	-0.82	-0.39
Rubber	-0.13	-0.15	0.15	0.13
Metal	0.61	-0.51	0.49	0.31
Wood	0.04	-0.21	0.3	-0.07
Paper	-0.12	-0.09	0.27	0.02
Textile	-0.15	-0.05	0.21	0.04
Ceramic	1.02	-0.22	0.06	-0.63
Glass	1.44	-0.19	0.71	-0.5
Other	-0.2	-0.23	-0.01	0.07
Mesoplastic	-0.25	0.54	-0.03	0.49

Table 5: Redundancy analysis for parallel transect beach (N=40) litter data (including mesoplastics) with log transformation on model: *rda*(Material abundance ~ Location + Beach type + Sediment + Clean up + Policy protection)

Variable	Df	Variance	F	P-value
Location	1	0.01	3.98	0.05*
Beach type	3	0.03	2.04	0.05*
Sediment	2	0.02	1.25	0.03*
Clean up effort	1	0.03	2.17	0.001***
Policy protection	1	0.01	0.89	0.11
Residual	30	0.16		

Significance scores: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.01. '' 1

RDA axes	Df	Variance	F	P-value
RDA 1	1	0.04	7.96	0.001***
RDA 2	1	0.02	4.22	0.09
RDA 3	1	0.01	2.35	0.59
RDA 4	1	0.01	1.03	0.99
RDA 5	1	0.00	0.57	1.00
RDA 6	1	0.00	0.35	
RDA 7	1	0.00	0.25	
RDA 8	1	0.00	0.16	
Residual	30	0.16		

Significance scores: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.01. '' 1

Table 6: Redundancy analysis for perpendicular transect beach (N=40) litter data (including mesoplastics) with log transformation on model: *rda*(Material abundance ~ Location + Beach type + Sediment + Clean up + Policy protection)

Variable	Df	Variance	F	P-value
Location	1	0.01	1.98	0.05*
Beach type	3	0.02	1.62	0.05*
Sediment	2	0.01	1.83	0.02*
Clean up effort	1	0.02	4.45	0.001***
Policy protection	1	0.01	1.55	0.11
Residual	30	0.09		

Significance scores: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.01. '' 1

RDA axes	Df	Variance	F	P-value
RDA 1	1	0.02	8.74	0.001*
RDA 2	1	0.01	5.11	0.09.
RDA 3	1	0.01	2.92	0.60
RDA 4	1	0.00	1.77	0.98
RDA 5	1	0.00	0.63	1.00
RDA 6	1	0.00	0.57	
RDA 7	1	0.00	0.37	
RDA 8	1	0.00	0.18	
RDA 9	1	0.00	0.11	
Residual	29	0.09		

Significance scores: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.01. '' 1

Table 7: Overview of marine litter policy, monitoring, coordination and data collation mechanisms.

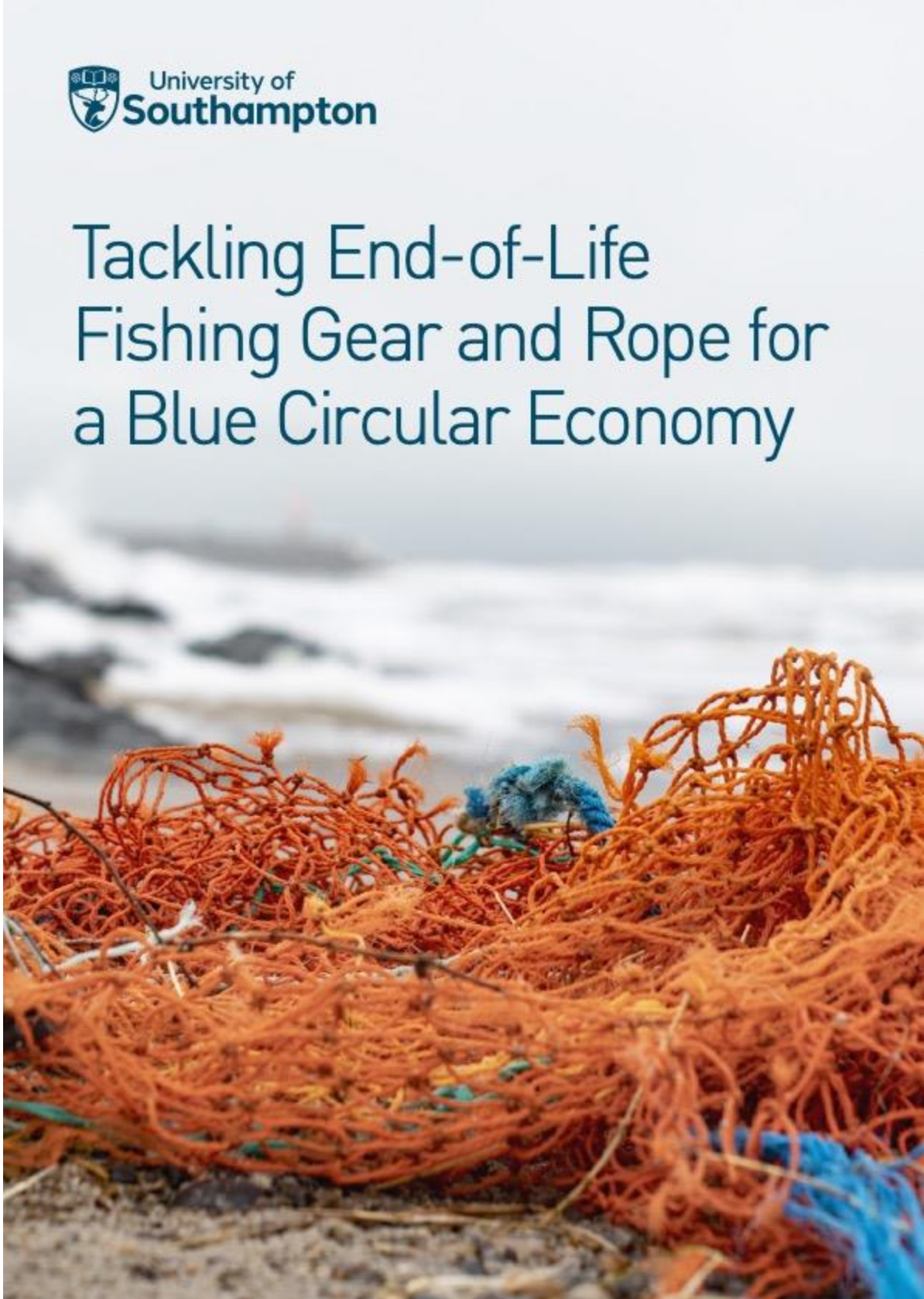
Monitoring, Coordination & Data Collation Mechanism	Goal	Targets	Policy Framework	Method	Litter quantification	Data platform	Includes citizen science
International							
UN SDG 14.1	Life Below Water, a goal focused on oceans that currently has 10 targets	By 2025, prevent and significantly reduce marine pollution of all kinds, in particular from land-based activities, including marine debris and nutrient pollution	2030 Agenda for Sustainable Development	"Global Manual on Ocean Statistics for Measuring SDG 14.1.1, 14.2.1 and 14.5.1" supports the range of methods as outlined by GESAMP (2019)	Items/km ²	SDG Data Portal	No
GPML	Protect the global marine environment, human well-being and animal welfare by addressing the global problem of marine litter and plastic pollution	Provide a multi-stakeholder platform to identify gaps, issues, share ideas, harness expertise and make a significant contribution to the 2030 Agenda, in particular SDG14.1	n/a	A series of phases will cumulate in a final version of the Digital Platform in June 2023	n/a	GPML Digital Platform	Yes
Litterbase	Provide descriptive statistics from over 3,000 scientific studies	n/a	n/a	Litter types contributing to the global composition of each marine compartment is calculated as the weighted means from all considered studies, irrespective of units	Data layers on a global map: items per km/km ² /m ³ /other and size class of litter nano/micro/macro	Litterbase	Yes
The Ocean Conservancy	Global monitoring of beaches through coastal clean-up events and a dedicated app (Clean Swell) for citizen science input	n/a	n/a	Citizen scientists record the distance, weight and items collected on a beach clean	Distance (miles/km) and weight (pounds/kg)	TIDES	Yes
Regional							
RSPs	Action-oriented programmes that implement region-specific activities, bringing together stakeholders including governments, scientific communities and civil societies	OSPAR adopted beach litter as a common indicator in 2013 and are aligning with other regional efforts e.g. EU MSFD (OSPAR, 2021)	Multilateral Environmental Agreements	Method for RSPs outlined by OSPAR (2010) guidance to monitor 100m parallel to the shore from the water's edge to the back of the beach	Items per unit length of coastline (items per m/km)	OSPAR Beach Litter Database, HELCOM map and data service	No
EU MSFD	Requires EU Member States to ensure that, by 2020 "properties and quantities of marine litter do not cause harm to the coastal environment"	Ban on SUPs (list of 6) and containers made of expanded polystyrene and oxo-degradable plastic, measures to reduce food and drink contact plastics, improve labelling, introduce EPR, achieve a 90% collection target for plastic bottles by 2029 (77% by 2025), re-design caps and use 25% of recycled plastic in PET bottles by 2025 and 30% from 2030	The MSFD provides legally binding requirements for implementing monitoring programmes for assessment of EU waters for GES. Marine plastic pollution is one of three major areas for the Strategy for Plastics, adopted by the Commission in 2018, which introduced the Directive on SUPs and Fishing Gear	In accordance with MSFD 'Guidance on Monitoring' Recommendation 3: Integrate already established monitoring programmes and relevant guidance, including RSC schemes. A "master list" of item categories is also provided by the TSG-ML	Items per unit length of coastline (items per m/km)	EMODnet	No
EU EEA	Fill data gaps and raise awareness about the problem of marine litter and the policy response	n/a	European policymaking	Clean-up initiative that weighs different litter materials	Total weight of litter for area covered	Marine LitterWatch	Yes
NOAA Marine Debris Monitoring and Assessment Project (MDMAP)	The MDMAP measures macro-sized marine debris (roughly the size of a bottle cap or larger) on shorelines and functions as a network of partnering organizations and volunteers	The ultimate goal is to develop more effective prevention and mitigation strategies to prevent the impacts of marine debris	Marine Debris Act (2006)	4 random transects perpendicular to the shore, 5m wide and 2m into the back of the beach, along a 100m stretch of beach	Prior to 2021 - items per 100m of shoreline	MDMAP dataset	Yes
CSIRO	Field sampling to measure (and mathematical modelling to estimate) the distribution and movement of plastic waste near urban centres, along waterways, on the coast and in the ocean	Design robust sampling plans tailored for each country involved to monitor a range of environmental compartments	n/a	3-6 transects (2m wide) that run from the water's edge to the vegetation near the coast	Items/m ²	n/a	Yes
National - UK							
Cefas	Conserve and manage marine and freshwater environments by forecasting ecosystem changes and hazards from toxins and disease	The target aims for an overall reduction in the number of visible litter items on coastlines. This has been supported by two surveillance indicators: (1) amount of litter on the sea floor, and (2) the amount of plastic in fulmar stomachs, which acts as a proxy for the amount of floating litter. Quantities of beach litter are also used to assess progress.	The UK Marine Strategy (2012), Defra's 25 Year Environment Plan and Food Strategy, UN SDGs	Beach data utilized from the MCS or method based on OSPAR (2010) is used for international projects	Items per 100m stretch of coastline from the strandline to the back of the beach	Links provided to the OSPAR Beach Litter Database	No
MCS	Provide UK monitoring of beaches through coastal clean-up events and citizen science input	Monitoring data from over 25 years helps support policy measures and measure their impacts	n/a	Annual beach clean and survey across the UK led by volunteers, based on the OSPAR (2010) approach	Items per 100m stretch of coastline from the strandline to the back of the beach	Need to contact to access data	Yes

Appendix III

Policy brief: Tackling end-of-life fishing gear and rope for a blue circular economy



Tackling End-of-Life Fishing Gear and Rope for a Blue Circular Economy



CONTEXT

Brexit leaves the United Kingdom (UK) with a unique opportunity for environmental innovation through the implementation of a set of national policy documents (25 Year Environment Bill; Resources and Waste Strategy; Industrial Strategy). Yet, statutory targets for waste reduction and recycling progress within the UK have largely been steered by more progressive European Union (EU) regulations (Waste Framework Directive 2008/98/EC).

The EU is shifting further toward circular economy design (Single Use Plastics [SUPs] Directive [EU] 2019/904; Port Reception Facilities [PRF] Directive [EU] 2019/883) and acts as a world leader in this regard, setting standards which the rest of the globe follows thanks to the power, strength and size of the single market. An agreement in principle exists to transpose aspects of the EU Circular Economy Package into UK law¹, however, until fully legislated there is a risk of falling behind on trade opportunities, economic benefits and environmental standards or obligations (e.g. eco-labels). Furthermore, evidenced success and ambitions in UK administrative waste management strategies and targets are not currently aligned (i.e. Wales – 75% municipal waste recycling by 2025; England – 65% by 2035). Ultimately, we need aligned, targeted responses and goals to ensure an efficient and successful transition to improved resource and waste management across industries.

This policy brief acts as an industry specific case study, covering key insights and recommendations for the improved management of end-of-life fishing gear (EOL FG) and rope within the UK. Abandoned, lost or otherwise discarded fishing gear (ALDFG), as termed by policy makers, is one of the most hazardous types of plastic pollution and of global concern. It is a direct threat to marine life, fisheries stocks,

maritime navigation and human safety, therefore improved management strategies and support for organisations establishing best practises are strongly encouraged.

In addition to these hazards, UK municipalities spend over £15 million each year removing beach litter each year. OSPAR (2019) found fishing related items one of the top three most common litter types recorded, while our UK wide marine litter survey in 2021 assessed 48 beaches, with fishing gear and rope items contributing 5 of the top 10 items recorded (Figure 1). Over half of these sites are under existing environmental policy protection, with hotspot areas generally the more remote beaches in proximity to fishing activity.

**Relevant UN
Sustainable
Development
Goals**



Photographer: Jo Morely

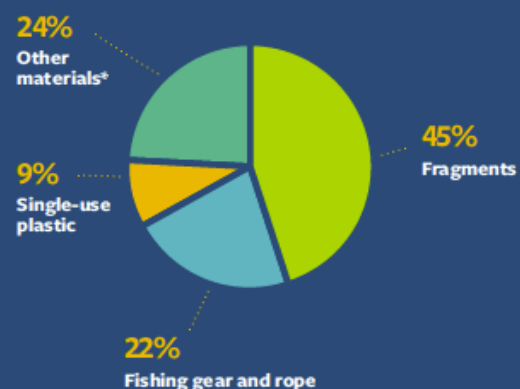
KEY INSIGHTS

- Although companies in the UK and EU assemble FG, most parts/materials are produced in other countries, and nets can be manufactured using nearly 700 different combinations of six raw polymer types and other materials (sometimes toxic, e.g. lead); an impossible endeavour for single unit recycling.
- Monitoring and enforcement by regional or national authorities at the sales and operations level is rare, thus allowing for general acceptance of using hazardous substances (as outlined by ECHA and REACH² regulations), providing little motivation to amend manufacturing or design processes.
- All ports within European Economic Area (EEA) states must provide port reception facilities (EU Directive 2000/59/EC; IMO Resolution MEPC³ 83[44]) and waste management plans. Yet, regionally specific operational and logistical difficulties (collection, segregation, cleaning, storage capacity, clear signage) and costs associated with recycling influence the economic benefits of material recovery. Subsequently, large unquantified volumes of FG, rope and other shipboard wastes are destined to subsidised landfill or incineration facilities.

KEY RECOMMENDATIONS

- A holistic approach is necessary to move towards improved management of EOL FG, which should include aspects such as targeted reductions of virgin plastics and incentives to help transition to recyclable or biodegradable/non-polymer/non-fossil fuel-based FG and rope materials. This process could be facilitated through a well-formulated hierarchy of needs analysis (e.g. commercial vs recreational vessels, or static vs mobile FG) and requires further research and stakeholder engagement from across the FG and rope material supply chain.
- The sharing of best practice, guidance and clear legal structure to exact waste segregation and cleaning of FG is urgently needed. In line with the revised PRF and SUPs Directives, port docking fees should cover the costs of disposing of all types of FG and rope in a responsible manner, with the support of equitable waste management strategies (including EPR schemes).
- Educational outreach programmes are key to raising environmental awareness and promoting the value of remanufactured FG products. Such incentives may increase cooperation (e.g. reporting lost gear as per IMO MARPOL Annex V/ FAO/ UNEP), encourage generational influences regarding sustainable practices, validate labour efforts and ensure the success of recycling schemes. Opportunities may be available to upskill and target new workers, but appropriate funds and recruitment drives are needed to overcome occupational skills shortages (such as deckhands with net-mending and rope splicing skills).
- Innovative funding mechanisms (e.g. Sycomore, rePurpose, Circular Action Hub, Plastic Credit Exchange), well-coordinated partnerships, engagement platforms and policies are required to support smaller actors and start-ups. To maximize recycling potential and improve tourism aesthetics, investment in small-scale waste storage infrastructure and management in ports is needed. Research and innovation to develop and test new materials and designs, which must be economically competitive, and of equal or improved durability and recyclability to current counterparts is also required.
- Market-based instruments and incentives that reward high sustainability in FG, or trialling of new technologies, could ease a transition toward improved management and more circular FG. For example, sustainability stamps that adhere to a comprehensively coordinated set of standards for FG (e.g. gear labelling, traceability, and accurate/ transparent information on all material components), or economic incentives (e.g. extra fishing quota, or market price gains for sustainably sourced fish).
- All four UK administrations must collaborate on circular economy policy to assist in the synchronicity of domestic legislation and the development of clear, well-aligned mechanistic frameworks. Communication should remain between UK regulatory agencies, European regulatory bodies and European Standards Organisations (e.g. Eurocords Technical Committee 3: Life Cycle Management and Circular Design of FG), as loss of access to such networks could lead to a reduction in expertise and specialist knowledge sharing, and may induce an international divergence in standards.
- Legislation mandating the use of recycled content will help support the recycling system, drive demand for recycled plastic and achieve climate goals. However, current policies only apply to certain types of packaging, which represents a small part of the plastics market. If such initiatives were considered across all types of manufacturing (such as the growing automotive, construction and energy industries) it could drastically improve the uptake of recyclate.

6,536 PIECES OF MARINE LITTER FOUND



*Non-SUP plastic items, clothes, glass, ceramics, metal and cardboard

Figure 2: Marine Litter Items Surveyed Across 47 UK Beaches

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