EMFF Operational Programme 2014-2020

Marine Biodiversity

Nephrops and Microplastics

EMFF 2014-2020

Marine Institute Report Series

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Specific Objective	UP1 SO1 - Reduction of the impact of fisheries and aquaculture on the marine environment, including the avoidance and reduction, as far as possible, of unwanted catch. UP1 SO2 - Protection and restoration of aquatic biodiversity and ecosystems. UP6 SO1 - Development and implementation of the Integrated Maritime Policy
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Our Vision

A thriving maritime economy in harmony with the ecosystem and supported by the delivery of excellence in our services.

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1. Introduction

Marine litter has been defined as "… persistent, manufactured or processed solid material that is discarded, disposed of or abandoned in the marine and coastal environment" with plastics being a major component/contributor to marine litter (Galgani *et al*. 2010, IUCN 2021). Plastic litter, once introduced into the marine environment can fragment into smaller plastic pieces known as microplastics (MPs) due to weathering and degradation (Thompson *et al*. 2004). MPs are defined as any synthetic solid particles of different shapes, with sizes ranging from 1 µm to 5 mm, of items that are of primary or secondary manufacturing origin, which are insoluble in water (Arthur *et al*. 2009, Frias and Nash 2019). MPs are problematic on a global scale and have been reported in all marine compartments explored to date (Bergmann *et al*. 2019, Frias *et al*. 2020, Pagter *et al*. 2020b, Pagter *et al*. 2020a). The persistent and ubiquitous nature of these contaminants in the marine environment has received particular attention in the last decade from the scientific community, stakeholders, policy makers, and the public (Fossi *et al*. 2018, Bessa *et al*. 2019, Alomar *et al*. 2020).

Due to their small size, MPs have been reported in a range of aquatic organisms from crustaceans to cetaceans (Wójcik-Fudalewska *et al*. 2016, Zhu *et al*. 2019, Hara *et al*. 2020). The behaviour of ingested MPs has been investigated in several laboratory studies, with researchers reporting that MPs may be egested or accumulated in the gastrointestinal tract (GIT) which have been shown to cause adverse effects in marine biota including a decrease in nutritional state, weight loss, energy depletion, blockages and toxicity (Besseling *et al*. 2013, Wright *et al*. 2013, Welden and Cowie 2016b, Devriese *et al*. 2017, Pannetier *et al*. 2020). The ubiquitous nature of MPs has led to an increased focus on commercial seafood species as there is potential for this contaminant to enter the human food chain (Smith *et al*. 2018, Hara *et al*. 2020). Several studies to date have reported MPs in the GIT of the Dublin Bay Prawn*, Nephrops norvegicus* and the surrounding sedimentary environment (Welden and Cowie 2016a, Martin *et al*. 2017, Hara *et al*., 2020, Cunningham *et al*. 2022).

The Dublin Bay prawn, *N. norvegicus* is one of the most commercially important species landed by the Irish Fleet, worth approximately €37 million in 2020 (Marine 2021). It is a benthic species, widely distributed on muddy substrates throughout the North-East Atlantic and Mediterranean Sea (Cau *et al*. 2020, Hara *et al*., 2020, Martinelli *et al*. 2021). These decapods are found at depths ranging from 20 – 800 m (Hill 2008) and are relatively sessile organisms, living in burrows of 20- 30 cm in depth, only emerging to forage and mate (Rice and Chapman 1971). They are opportunistic scavengers that consume a variety of organisms including polychaetas, molluscs, crustaceans, and echinoderms (Parslow-Williams *et al*. 2002, Murray and Cowie 2011, Carreras-Colom *et al*. 2022).

Kingdom: Animalia

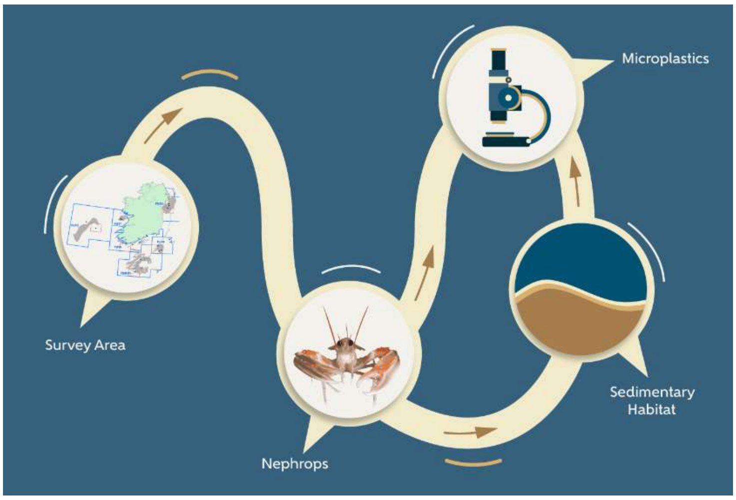
Phylum: Arthropoda

Class: Malacostraca

Figure 1. Classification of the Dublin Bay Prawn, *Nephrops norvegicus* (Credits: Haleigh Joyce, 2022)

Due to their high economic value, spatial distribution, and ecological relevance *N. norvegicus* have the potential to be used as a bioindicator for MP contamination (Fossi *et al*. 2018, Cau *et al*. 2019) and can be used to provide information to guide policy makers and environmental managers. This research focuses on the MP loadings in *N. norvegicus* and the exploration of a potential relationship with their surrounding sedimentary habitat within six primary *N. norvegicus* fishing grounds in the North East Atlantic. While *N. norvegicus* has been documented to ingest MPs, this research builds on the knowledge base through exploring the ingestion and retention times of MPs of varying sizes. This research proposes a pan-European monitoring programme to detect MP abundances and changes in levels through the use of *N. norvegicus* as a potential bioindicator for MP contamination.

2. Microplastic loadings in *Nephrops norvegicus* and surrounding habitat in the North East Atlantic



This chapter is a verbatim reproduction of the following published manuscript:

Joyce, H., Frias, J., Kavanagh, F., Lynch, R., Elena Pagter, White, J., Nash, R., 2022. Plastics, prawns, and patterns: Microplastic loadings in Nephrops norvegicus and surrounding habitat in the North East Atlantic. Science of the Total Environment, 154036. DOI: 10.1016/j.scitotenv.2022.154036

Supplementary information to this paper can be found in Appendix I

Research highlights:

- *Nephrops norvegicus in combination with habitat as a potential monitoring tool for microplastics.*
- *Low microplastic levels were recorded in N. norvegicus, indicating that microplastics do not bioaccumulate.*
- *Microplastic contamination was assessed in Nephrops norvegicus and sediment from six primary fishing grounds.*
- *Microplastic types and colours from organisms were similar to those retrieved from the surrounding sediment.*
- *Mean abundance of microplastics recorded in N. norvegicus (n=600) was 2.20 ± 2.47 items per individual.*

Keywords:

Microplastics, North-east Atlantic, *Nephrops norvegicus*, Sediments, Marine Strategy Framework Directive.

2.1 Abstract

The presence of microplastics (MPs), a contaminant of emerging concern, has attracted increasing attention in commercially important seafood species such as *Nephrops norvegicus*. This species lend themselves well as bioindicators of environmental contamination owing to their availability, spatial and depth distribution, interactions with seafloor sediment and position in the ecosystem and food chain. This study assesses the abundance of MPs in *N. norvegicus* and in benthic sediments across six functional units in the North East Atlantic. Assessment of the relationship between MP abundance in *N. norvegicus*, their biological parameters and their surrounding environment was examined. Despite the lack of statistical significance, MP abundances, size, shape, and polymer type recorded in *N. norvegicus* mirrored those found in the surrounding environment samples. The three main polymers identified in both organisms and sediment were polystyrene, polyamide (nylons), and polypropylene. The level of MP contamination in *N. norvegicus* could be related to local sources, with relatively low abundances recorded in this study for the North East Atlantic in comparison to other regional studies. Furthermore, larger organisms contained a lower abundance of MPs, demonstrating no accumulation of MPs in *N. norvegicus*. Based on the results of this study, data on MP ingestion could be used to study trends in the amount and composition of litter ingested by marine animals towards fulfilling requirements of descriptor 10 of the Marine Strategy Framework Directive.

2.2 Introduction

The global production of plastic has increased exponentially since the inception of the plastics industry in the 1950's. Up until 2017, a total of 9.2 billion tonnes had already been produced (Plastic Atlas, 2021), with Europe's production alone reaching almost 55 million metric tonnes in 2020 (Tiseo, 2022). Plastic is an important material in modern society (Patrício Silva *et al.*, 2020) which has substantially improved our quality of life (Plastics Europe, 2020). The amount of plastic waste produced, which has been rising over time, is expected to more than double by 2050 (Geyer *et al*., 2017; Lebreton and Andrady, 2019). Furthermore, during the COVID-19 pandemic, the use of single-use plastics increased and therefore these predictions will likely be exacerbated (Benson *et al.,* 2021; Patrício Silva *et al.*, 2021). The release or incorrect disposal of these materials into the environment will likely have negative impacts (Stefatos *et al.*, 1999; Gregory, 2009; Plastics Europe, 2020).

The abundance of plastic pollution has led to a large accumulation of secondary microplastics (MPs) within the marine environment (Isobe *et al.*, 2019), resulting from both degradation and fragmentation of these larger plastics (Kershaw, 2015). MPs are introduced into the marine environment from a variety of different sources and pathways (Rochman *et al.*, 2019). MP sources from land include the agricultural sector (Rehm *et al.*, 2021), tourism (Retama *et al.*, 2016), personal care products (Fendall and Sewell, 2009), domestic waste (Siegfried *et al.*, 2017), and transport (Evangeliou *et al.*, 2020) but can also originate from marine sources such as fisheries (Deshpande *et al.*, 2020) and shipping (Ng and Obbard, 2006). This accumulation of MPs and its expected increase in the marine environment demonstrate a need to monitor the environment to assess the future socio-economic and environmental impacts.

MPs are ubiquitous and have been identified in every ecosystem explored to date, including intertidal and subtidal sediments (Wang *et al.*, 2019; Alvarez-Zeferino *et al.*, 2020), seawater (Frias *et al.*, 2020), the Arctic (Kanhai *et al.*, 2020) and the Antarctic (Jiang *et al.*, 2020) regions. MPs have even been recorded from the top of Mount Everest (Napper *et al.*, 2020) and in the Marianna Trench, a single use plastic bag was identified at a depth of *ca*. 10,900m (Chiba *et al.*, 2018).

MPs are considered potentially hazardous due to their physical and chemical composition and persistent nature, having the ability to affect both aquatic habitats and organisms (Rochman *et al.*, 2013; Jambeck *et al.*, 2015). MPs have been ingested by many organisms such as fish (Lusher *et al.*, 2015), seabirds (Acampora *et al.*, 2016), gastropod molluscs (Doyle *et al.*, 2019) and decapod crustaceans (Hara *et al.,* 2020; Cau *et al.*, 2019). Marine biota and human exposure to MPs are considered key research topics in recent years (Hossain *et al.*, 2020).

Bioindicators can be used to assess environmental health (Holt and Miller, 2011). Mussels (*Mytilus sp.*) have been acknowledged as a key bioindicator species under the Mussel Watch Programme (Beyer *et al.*, 2017) and as a potential bioindicator for MP contamination in the environment (Li *et al.*, 2019). MPs are documented in many marine organisms, and more recently there is an increasing number of studies with a sufficient baseline data for suitable representation of MP loadings at the metapopulation/population level, as suggested by Hermsen *et al.,* (2018).

Nephrops norvegicus (Linnaeus 1758) is a decapod crustacean commonly referred to as the Dublin Bay Prawn or the Norway Lobster found living in muddy bottom environments in deep waters (Welden *et al.,* 2015*;* Cau *et al.,* 2020). In Europe, *N. norvegicus* are considered to be of high economic value, for example within the Irish fishing industry the 2018 landings were estimated to be worth more than €56 million (Marine Institute, 2020a). Despite this, few studies focus on the ingestion of MPs in this commercial species in Ireland (Hara *et al*., 2020).

N. norvegicus are opportunistic feeders with a diet mainly composed of molluscs, echinoderms, polychaetes and crustaceans (Murray and Cowie, 2011; Welden *et al.*, 2015) with consumption of non-food materials also recorded (Parslow-Williams *et al.*, 2002). They have the capability to ingest solid particles of up to 20 mm in length and 4 mm in width (Yonge, 1924). This non-selective feeding behaviour, and possibly burrowing habits are potential reasons for the presence of MPs in *N. norvegicus* (Murray and Cowie, 2011; Andrades *et al.*, 2019).

There are previous studies that identified MPs in *N. norvegicus,* for example Martinelli *et al.,* (2021) looked at relatively low number of the species, Cau *et al.,* (2020) looked at a localised area, Welden and Cowie, (2016a) didn't include a digestive process. This study is novel in that it takes a more comprehensive methodological approach exploring two environmental matrices covering an extensive geographical area incorporating key *N. norvegicus* Irish fishing grounds in the North East Atlantic. *N. norvegicus* are known to feed close to their burrows, illustrating the potential for MP contamination of wild caught organisms from their surrounding environment (Cau *et al.*, 2019). A study investigating MP ingestion of *N. norvegicus* from three locations in the North Atlantic Ocean (North Sea, North Minch and the Clyde Sea) recorded a large variation in the presence of MPs within the organisms (28.7%-84.1%), suggesting a possible link between the MPs available in surrounding habitat and the amount of MPs ingested by organisms (Welden, 2015). Furthermore, (Welden and Cowie, 2016b) discovered that ingestion of polypropylene fibres may negatively affect the growth and nutritional state of *N. norvegicus,* with prolonged exposure over time potentially leading to secondary effects such as mortality and decreased fecundity, with contradictory results from (Devriese *et al.*, 2017) illustrating that MP ingestion did not affect nutritional state of *N. norvegicus* during 3 weeks of exposure.

The European Food Safety Authority (EFSA) highlighted the need for assessing and monitoring MPs as a seafood contaminant and the potential effects it may have on human health (EFSA, 2016). A 2019 report which assessed European's awareness of food safety topics highlighted that 48% of respondents were aware of MPs in food, illustrating an increasing public concern for plastic contamination and food safety (EFSA, 2019). There is currently no legislation in place regulating MPs as potential contaminants of seafood (Rainieri and Barranco, 2019).

The primary aim of this study was to assess the abundance and characteristics of MPs in *N. norvegicus* and their associated benthic habitat in six functional units in the North East Atlantic. The authors hypothesized that MP abundance in organisms and benthic sediment varies between Functional Units (FU's), with higher MP abundances expected with increasing proximity to shore. In determining a baseline this research further explored if MP abundances were correlated with sex, size, moult stage and presence of the parasitic dinoflagellate *Hematodinium* spp. Furthermore, this study assessed whether *N. norvegicus* would also be suitable as a bioindicator for MPs. The results may inform policy makers and potential future monitoring in respect of the Marine Strategy Framework Directive (EC, 2008).

2.3 Materials and Methods

2.3.1 Study Area

Areas of suitable seafloor around Europe comprising habitat for *N. norvegicus* have been designated into specific fishing grounds, referred to as functional units (FUs) each with a designated number. FU's around Ireland fall within the International Council for the Exploration of the Sea (ICES) Subarea 27.7 (Irish Sea, West of Ireland, Porcupine Bank, Eastern and Western English Channel, Bristol Channel, Celtic Sea North and South, and Southwest of Ireland - East and West) (ICES, 2012) . Samples of wild *N. norvegicus* populations were collected from the six primary fishing grounds, namely: (i) Irish Sea West (FU15) (ii) Porcupine bank (FU16) (iii) Aran prawn ground (FU17) (iv) SW and SE coast (FU19) (v) Labadie, Jones, and Cockburn (FU20-21) (vi) Smalls (FU22) (Figure 1). These areas are defined as primary fishing grounds for *N. norvegicus* by the Irish Marine Institute owing to reviews of fishing activity, with stocks surveyed annually Under-Water Television Surveys (UWTV) (Marine Institute, 2020b).

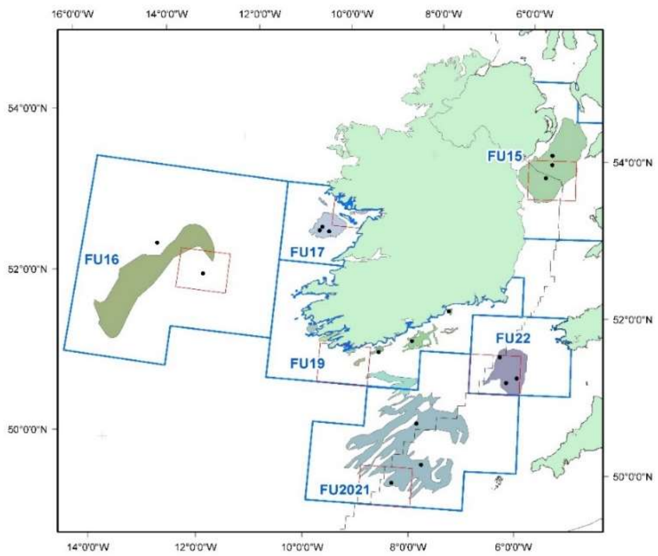


Figure 1. Designated Functional Unit extensions are delimited in blue: (i) Irish Sea West (FU15) (ii) Porcupine Bank (FU16) (iii) Aran Prawn Ground (FU17) (iv) SW and SE coast (FU19) (v) Labadie, Jones, and Cockburn (FU20-21) (vi) Smalls (FU22). Shaded areas correspond to suitable habitat and UTVW FU survey grounds; ICES Statistical Rectangles outlined in red represent sampling sites for *N. norvegicus* and black dots represent benthic sediment sampling sites.

2.3.2 Nephrops norvegicus

Collection

All *N. norvegicus* samples were provided by the Irish Marine Institute and were obtained from commercial fishing vessels between March and October 2020. Sample collection was carried out within the six pre-established prawn grounds using standard commercial fishing gear, caught in compliance with EU fishing regulations. The individuals collected were representative of a commercial catch. *N. norvegicus* samples were subsequently frozen at -20 °C (Hermsen *et al.*, 2018) until further processing.

Laboratory analysis

Organisms were defrosted at room temperature and the exterior rinsed using ultra-pure water (ELGA PURELAB Option-R 7 BP water purification system, 18 MΩ, 0.2 µm POU filter). The sex, total length (TL), physical damage, carapace hardness, moult stage and presence of the dinoflagellate parasite *Hematodinium* spp. were recorded for each specimen, prior to dissection. TL was measured from the tip of the rostrum to the posterior edge of the telson, sex was determined by the structure of the sexual pleopods (Farmer, 1974) and females were also identifiable by the presence of external eggs (Eiríksson, 2014).

Carapace condition and moult stage of each organism was determined based on the methodology by Milligan *et al.,* (2009). Carapace condition was determined by the hardness of the individual's cephalothorax, divided into three categories, namely, (a) Hard: "if there was no noticeable give in the exoskeleton when squeezed behind the eyes"; (b) Soft: "if the squeezing caused clear distortion"; and (c) Jelly: "when the entire exoskeleton was very soft and gave no resistance to pressure" (Milligan *et al.*, 2009). The moult stage of each organism was based off the same categories, identifying intermoult stage organisms to be hard; late intermoult organisms with removed calcium from the exoskeleton or newly moulted stage but are no longer jelly to be soft, and very recently moulted organisms to be jelly (Milligan *et al.*, 2009; Murray and Cowie, 2011).

The physical damage observed on the external body of the *N. norvegicus* was based on a damage index proposed by Ridgway *et al.,* (2006) which categorises the structural damage caused to the specimen on claws, limbs, eyes, and soft tissue into three categories (a) no damage, (b) lightly damaged, and (c) heavily damaged.

Two methods were used to detect the presence of *Hematodinium* spp. for each individual. Firstly, a colour diagnostic method provides a fast assessment of advanced stages of infection, where parasite infestation can be identified by a vivid dull orange colouration of the carapace (Tärnlund, 2000; Stentiford *et al.*, 2001). Secondly, a pleopod method, requires the removal of a pleopod to be examined under a low light stereomicroscope (Olympus SZX7) at 40x for presence of dense aggregations of the parasite, appearing as darkened areas. Accumulation of parasitic material was then classified to stage of infection, 0-4, with 0 being uninfected and stages 1-4 patently infected (Field and Appleton, 1995; Tärnlund, 2000).

Microplastic analysis

Digestive tracts, consisting of the foregut, midgut, and hindgut, once removed, were immediately transferred to decontaminated labelled jars. Digestion of the digestive tract was carried out using a 10% potassium hydroxide (KOH) at 40 °C for 48 hours, as recommended by Hara *et al.,* (2020). The resulting digestate was filtered using a vacuum pump (VCP130) through 47 mm Whatman® (GF/C) glass microfiber filter paper (1.2 µm particle retention). The filter was then transferred onto a labelled petri dish for visual examination under a stereomicroscope Olympus SZX7. The particles that were identified as possible MPs were transferred onto blank sterile petri dishes where photographs and measurements were taken for MP colour, size and for polymer characterisation (Kanhai *et al.*, 2017). The MPs were counted, measured, and photographed using Olympus CellSens® software.

Types of MPs recorded were based on the identification schedule of Frias *et al.,* (2019) and size ranges (1 µm to 5 mm) applied based on the definition of (Frias and Nash, 2019). A Bruker Hyperion 2000 series FT-IR Microscope with a MCT (mercury cadmium-telluride) detector was used to identify the MP Polymers. Sample spectra were collected in transmission mode in 128 scans (minimum), with a spectral resolution of 4 cm^{-1}, in a wavenumber range of 4000–400 cm^{-1}. In addition, background spectra were measured with the same parameters prior to scanning the MP samples (Kanhai *et al.*, 2017). The JPI Oceans BASEMAN project FTIR polymer library was used for polymer identification.

2.3.3 Sediment

Collection

Sediment samples were provided by the Irish Marine Institute, which were collected between June 2020 and March 2021 from scientific surveys. All benthic sediment sampling occurred in waters at depths between 38-630 m. A Day Grab was deployed to collect benthic sediment samples for MP and granulometric analysis. Sediment samples were taken at each of the six primary fishing grounds around Ireland (Figure 1). Sediment samples were collected at 3 stations from five of the functional units (FU15, 17, 19, 20-21, and 22), while only two stations were achieved for FU16. Two replicate sub-samples were taken from each grab/sampling station (*n*=34). Furthermore, a single sample for granulometric analysis was collected from each station. All sediment samples were taken from the top 5 cm were placed into decontaminated glass jars with metal lids. All sediment samples were frozen at -20 °C until processing.

Laboratory analysis

Granulometry

Granulometry was used to determine the sediment composition and methodology was carried out as recommended by Pagter *et al.,*(2018). The sediment samples were defrosted and homogenized prior to being place in the oven to dry at 105 °C for 24 hours. Dried sediment (35 g) was weighed out, transferred into a glass beaker with 6% hydrogen peroxide (H_2O_2) (100 mL) was added and left for 12 hours to stand in the fume hood. The surplus H_2O_2 was washed out through a 63 µm sieve, and the sample retained in the sieve was washed back into the beaker where 10 mL of 10% sodium hexametaphosphate ($Na_6P_6O_{18}$) was added and allowed to stand for a further 12 hours. The sediment sample was washed again and left to dry for a further 24 hrs at 105 °C. Once dried, an automated column shaker (Endecotts Octagon Digital Sieve Shaker AAR 3915A) with a range of graduated sieves from 2 mm to 63 µm was used to separate sediment. The weight of sediment retained in each sieve was recorded using a Ohaus Adventurer scale. The silt/clay component was recorded based on comparisons of the initial sediment weight and entered into Gradistat® (version 8.0) software to distinguish the sediment composition.

Loss on Ignition

Loss on Ignition (LoI) was carried out to estimate the organic matter content within the sediment and methodology was carried out as recommended by Pagter *et al .,* (2018). The sediment samples were defrosted and homogenized prior to being place in the oven to dry at 105 °C for 24 hours. A subsample of the dried sediment was placed into a pestle and mortar and was crushed into a fine powder. Five grams of fine powdered sediment was baked in a furnace at 450°C. After 6 hours, the sample was removed from the oven and left in a desiccator to cool. The subsample was reweighed and the difference between the initial weight was recorded.

Microplastic analysis

Sediment was removed from the freezer and washed using ultra-pure water into aluminium trays and dried in an oven at 40 °C for approximately seven days. The dry sediment was weighed and placed into decontaminated jars. MPs were extracted from the sediment matrix using a density separation method using Sodium Tungstate Dihydrate ($Na_2WO_4 \cdot 2H_2O$) solution (41% w/v; 1.4 g/cm^3) as recommended by (Pagter *et al.*, 2018). Sodium tungstate solution was added to the sediment (3:1 ratio) (Claessens *et al.*, 2013). The mixture was stirred for 5 minutes with a stainless-steel stirrer, covered with aluminium foil to prevent contamination, and left to settle for 24hrs to allow for the settlement of the silt/clay component. Following the settling period, the supernatant containing floating MPs was pipetted off using a glass pipette and vacuum filtered using a vacuum pump (VCP 130) through a 47 mm Whatman® (GF/C) glass microfiber filter paper. Once the supernatant was filtered, the walls of the filtration device were rinsed using sodium tungstate dihydrate solution to avoid dilution of the solution, and to obtain any particles left on the walls of the funnel. The filter was then transferred onto a labelled petri dish for visual examination and sorting of MPs was performed under a stereo microscope connected to a camera with Olympus CellSens® software. This procedure was repeated three times for each sediment sample.

Classification of MP types, sizes and polymer composition followed that for *N. norvegicus* samples (see Section 2.2).

2.3.4 Contamination control

Cross–contamination was reduced by using a 100% cotton lab coat and nitrile gloves at all times (Pagter *et al*., 2018). Wearing of synthetic clothing under lab coat was avoided (Hermsen *et al.*, 2018). Decontamination of glassware was carried out using dilute (10%) Nitric Acid (HNO_3), followed by rinsing three times using ultra-pure water and left to dry upside down to avoid -accumulation of airborne particles. All surfaces were cleaned prior to use. Air controls were used every day during all stages of processing. Procedural blanks were carried out on ultra-pure water, sodium tungstate and potassium hydroxide to monitor potential contamination. The contamination quality control for the microplastic analysis in biota carried out in this study was assessed according to the criteria set out by (Hermsen *et al.*, 2018) and recorded a good score of 17/20.

2.3.5 Data analysis

All statistical modelling was performed in Minitab version 18 and RStudio version 4.1.1 software. Descriptive statistics and tests for normality were conducted on all data sets to determine whether parametric or non-parametric statistical analyses were appropriate. MP abundances were analysed using a Kruskal Wallis test for analysis of variance, followed by Dunn's test for multiple comparisons. A correlation analysis (Spearman Rank Correlation) was performed to examine the relationship between the abundance of MP and physical characteristics (body weight, total length, condition, moult stage, and sex of the tested samples. A correlation analysis (Spearman Rank Correlation) was also performed to examine the relationship between MP abundances in *N. norvegicus* and in the sediment within FU's. The significance level for all statistical tests was set at α = 0.05.

2.4 Results

The sex of the *N. norvegicus* (*n*=600) were identified as 52.3% female and 47.7% male. Out of the 600 individuals measured, 96.3% were assessed to be within the size at onset of sexual maturity (SOM), which is estimated to be 23.2 to 27.6 mm Carapace Length (CL) in females and 25.9 to 31 mm CL in males (McQuaid *et al.*, 2006). Total length (TL) ranged between 61.7 and 145.7 mm, with an average of 95.55 ± 14.01 mm. Almost half of the investigated individuals (47.84%) were observed to have a hard carapace condition, which is assumed to be at the intermoult stage. The organisms with a soft carapace condition represented 27.83% of the sample, which is assumed to be at late intermoult, or recent moult and the jelly organisms represented 24.33% of the sample, which is assumed to be at the very recent moult stage.

2.4.1 Microplastics and *N. norvegicus*

A total of 1,322 particles were extracted from the digestive tracts of 600 *N. norvegicus,* from 6 FU's in the North East Atlantic, with an average of 2.20 ± 2.47 MP items per individual. Of these samples, 430 out of 600 individuals (*c*. 72%) had ingested at least 1 MP particle.

Samples collected from the Western Irish Sea (FU15) exhibited the highest MP abundance, with an average of 3.66 ± 3.47 items per individual, while the lowest abundance was recorded in the Porcupine Bank (FU16) with 0.80 ± 1.21 items per individual (Figure 2). The FU's furthest from shore had the smallest abundance of MPs (FU16 and FU20-21), while those in the proximity or within the Western Irish Sea had the highest abundance (FU15 and FU22), however the SE and SW Coasts of Ireland (FU19) which is the closest site to shore recorded a lower abundance of MPs. Procedural blanks and air control contamination recorded while processing was minimal (0.38 ± 0.49 and 0.27 ± 0.45 respectively); therefore, no corrections were made to the analysis.

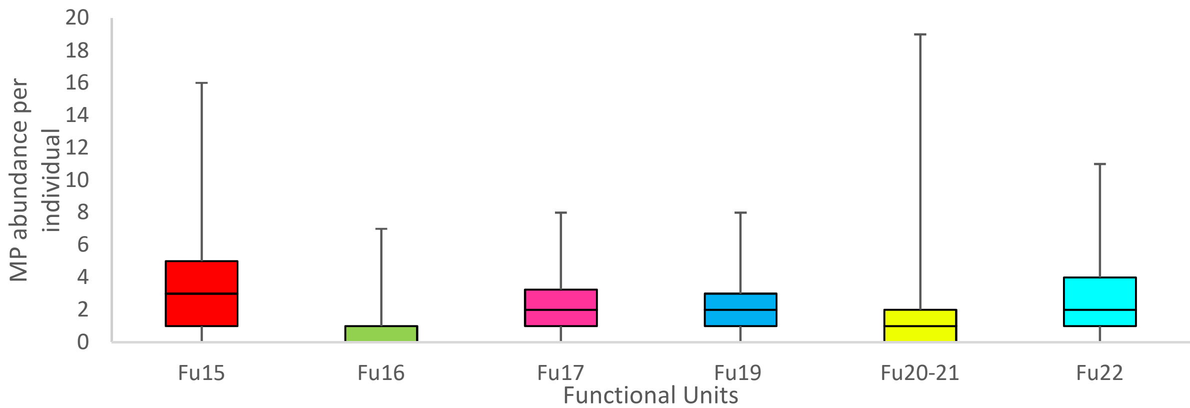


Figure 2. Boxplot showing the range in the abundance of MPs extracted from the digestive tracts of *N. norvegicus* at each Functional Unit (FU) (*n*= 100; N=600). Boxes represents the first and third quartile, middle bar the median and error bars maximum and minimum values.

The percentage of MP occurrence for each sampling station is presented in Table 1, where samples from the Aran Prawn Grounds (FU17) and the Western Irish Sea (FU15) recorded the highest percentage of individuals with MP's (84% and 82% respectively), while the lowest recorded was at the Porcupine Bank (FU16) (42%). The abundance of MPs ranged from 1 to 19 items per individual, with the highest abundance recorded from the Western Irish Sea FU15 (*n*=366) and the lowest recorded at the Porcupine Bank FU16 (*n*=80).

Table 1. Variation in MP occurrence and abundance at each Functional Unit (FU) and the proportion of individuals at each site (*n*=600) that recorded MPs. A post-hoc Dunn's test indicated significant differences between FU's indicated here as letters (A, B, C, D).

Sampling station (prawn grounds)	Total MP's recorded	Maximum MP count recorded	% Containing MP's	Median MP's recorded
The Western Irish Sea (FU15)	366	16	82	3 [A]
Porcupine Bank (FU16)	80	7	42	0 [B]
Aran Prawn Grounds (FU17)	231	13	84	2 [A, C]
SE and SW Coasts of Ireland (FU19)	215	8	77	2 [C]
Labadie Jones and Cockburn (FU20-21)	156	19	64	1 [D]
The Smalls (FU22)	274	11	81	2 [A, C]

FU16 and FU 20-21 were significantly different from all FU's. FU16 had the lowest number of MPs recorded. FU15 was significantly different from all FU's apart from FU22 and FU17. FU15 had the highest MP abundance followed by FU22.

Two main categories of MPs were recorded, with the majority identified as fibres (98.2%) and the remainder fragments (1.8%). Fibres ranged in length from 45 µm to 13.34 mm with one outlier measuring 53.88 mm, giving an average length of 1.43 mm. The most common size recorded was <1 mm (51%). Results show that 97.4% of all extracted MPs were within the defined size of MPs (>1 µm and <5 mm), while the rest consisted of particles >5 mm (2.6%) highlighting the presence of macroplastics among extracted particles.

A range of colours of MPs were extracted (Figure 3), with blue (62.6%) being the most prevalent, followed by black (8.8%), red (8.3%), grey (7.4%), transparent (4.8%), and other (8.2%), which included colours such as green, pink, purple, orange, multicoloured, yellow and white.

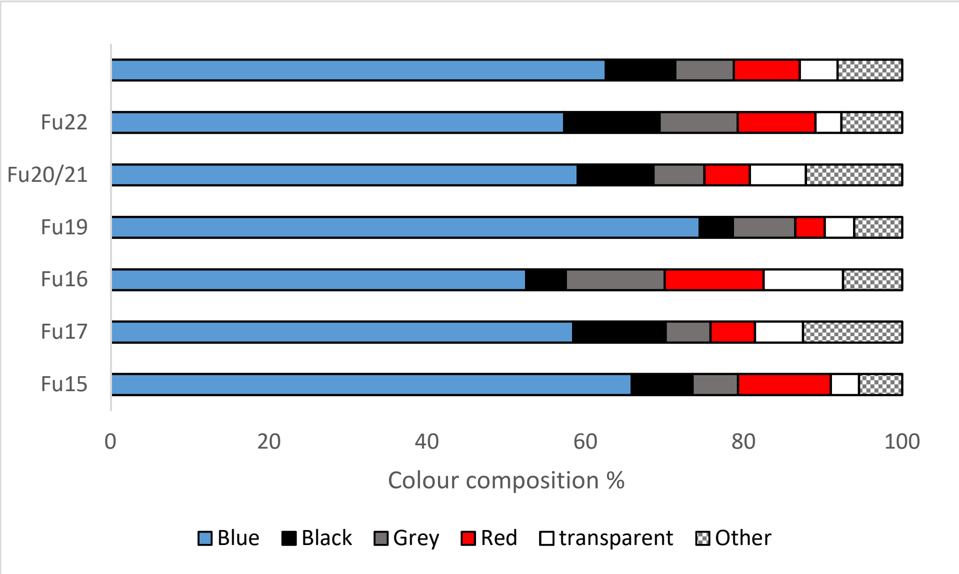


Figure 3. Colour composition of MPs in *N. norvegicus* across Functional Units (*n*=1322).

A subsample of MPs (*n*=367, 27.8%) was randomly selected for polymer identification, to include the factors sex, moult stage and length. The most common particles identified in the digestive tract of *N. norvegicus* were Polystyrene (PS), Nylon (polyamide) (PA), Polypropylene (PP) and Polyester. These polymers were recorded from all the FU's and combined they made up 36% of the MP particles analysed.

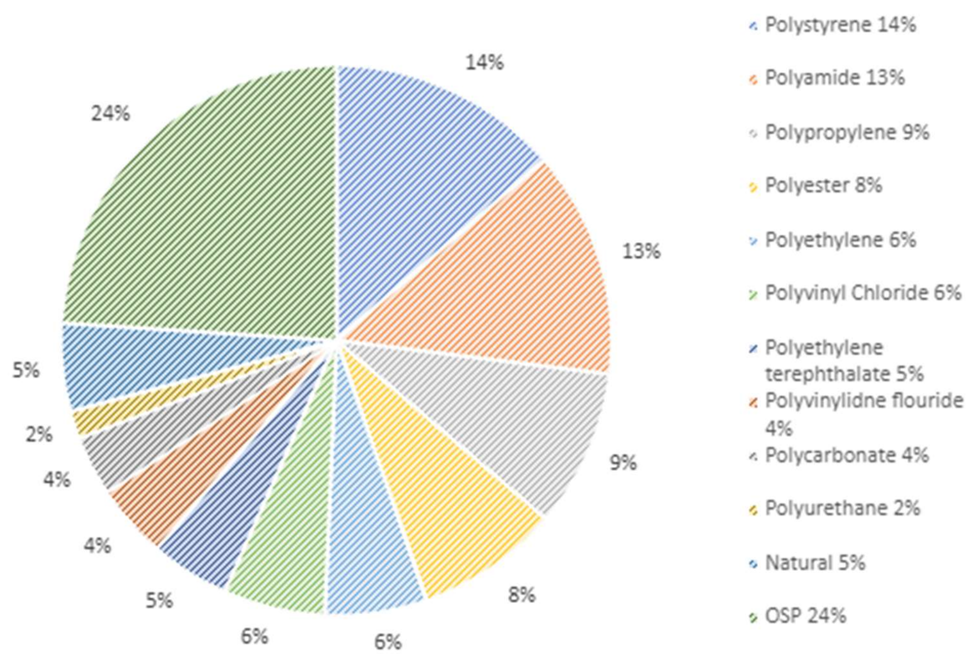


Figure 4. Polymer composition (%) of MPs ingested by *N. norvegicus* across all 6 Functional Units (FU's). The main plastics are categorised by resin types according to (Plastics Europe, 2019) in conjunction with Other Synthetic Polymers (OSP) and natural fibres e.g., cotton and linen.

N. norvegicus characteristics such as total length, sex, weight, and moult stage were examined for differences in MP abundance. In an overview of all FU's the smaller individuals (<82 mm) were recorded to have a higher MP abundance in comparison to larger individuals (>127 mm) (Figure 5). While no statistical significance was recorded (Spearman's correlation; p=0.297; n=430, excluding zero values) an inverse relationship was observed. An individual analysis on each FU showed that FU15 and FU16 had statistically significant relationships between TL and MP abundance (R_s = -0.236, p = 0.033 and R_s = 0.439, p = 0.004 respectively). The body weight for *N. norvegicus* was examined in one FU (FU16) with a mean of 21.85 ± 11.1 g, and maximum and minimum values equivalent to 69.99 g and 6.89 g, respectively. A Spearman's correlation analysis between body weight (g) and MP abundance indicated a positive correlation between the variables (R_s = 0.346, p-value <0.001).

Individuals with a hard carapace condition contained a mean of 2.03 ± 1.97 items per individual, soft carapace individuals 2.10 ± 3.01 items per individual and Jelly carapaces 2.24 ± 2.64 items per individual. Correlation analysis indicated no significant association between number of MPs present and carapace condition (Spearman's rank; p=0.908).

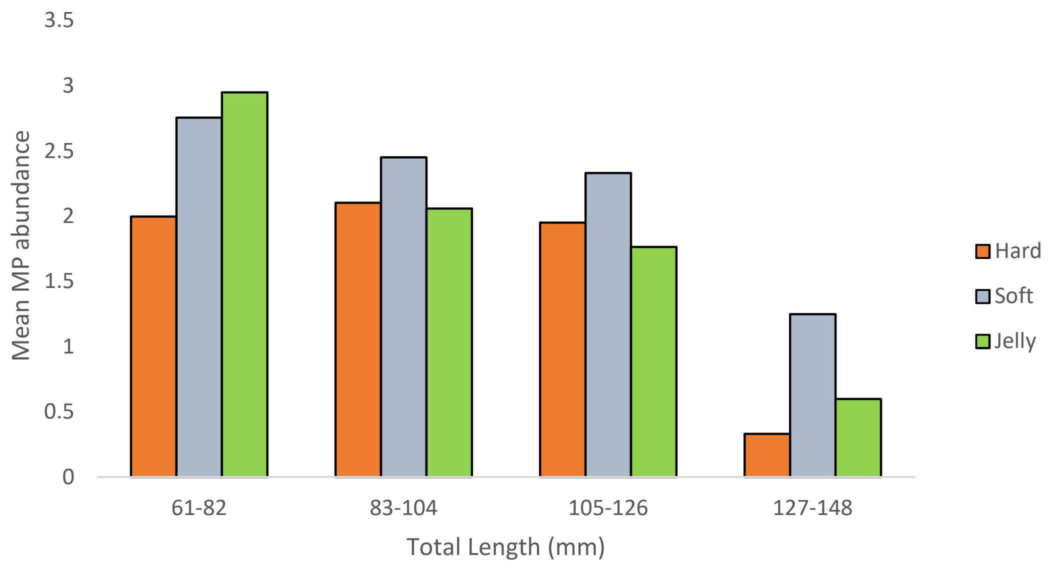


Figure 5. Mean abundance of MPs in relation to total length and carapace condition.

Females had a higher MP abundance (*n*=786) than males (Figure S1) with a mean of 2.47 ± 1.37 items/individual and males 1.88 ± 0.61 items/individual (*n*=536). Spearman correlation between sex and MP abundance (R_s=0.105) indicated a trend between the variables. An individual analysis on each FU showed that FU15 and FU22 had statistically significant relationships between sexes and MP abundance at the α = 0.05 level (p<0.001 and p=0.016 respectively), indicating that sex has an impact on MP ingestion for these FU's.

Almost half (47.9%) of dissected *N. norvegicus* showed heavy external damage as classified by (Ridgway *et al.*, 2006), while 34.8% were lightly damaged and 17.3% had no damage. Following the colour diagnostic method, 9% of examined *N. norvegicus* were infected with *Hematodinium* spp. across the six FU's (Figure S2), with FU20-21 having the highest rate and FU16 the lowest. Correlation analysis between *Hematodinium* spp. presence and MP abundance showed a positive correlation (R_s=0.063) but was not statistically significant (p=0.125).

The pleopod method of detecting *Hematodinium* spp. infection (Field and Appleton, 1995) showed a larger prevalence of the parasite. Using the index, infections were ranked into stages 0 to 4. Results showed that 54.8% were uninfected (stage 0), 39% were stage 1, 6% stage 2, 0.2% stage 3 and 0% at stage 4 (Figure S3). As with the colour method, FU 20-21 had the highest level of infection and FU16 the lowest, with correlation analysis to MP abundance, again indicating no significant association (p=0.586).

3.4.2 Microplastics and Sediments

A total of 104 MPs were recorded in 4 kg of dry weight (d.w.) sediment from the six FU's, with a mean abundance of 2.99 ± 1.80 microplastics/100 g dry sediment.

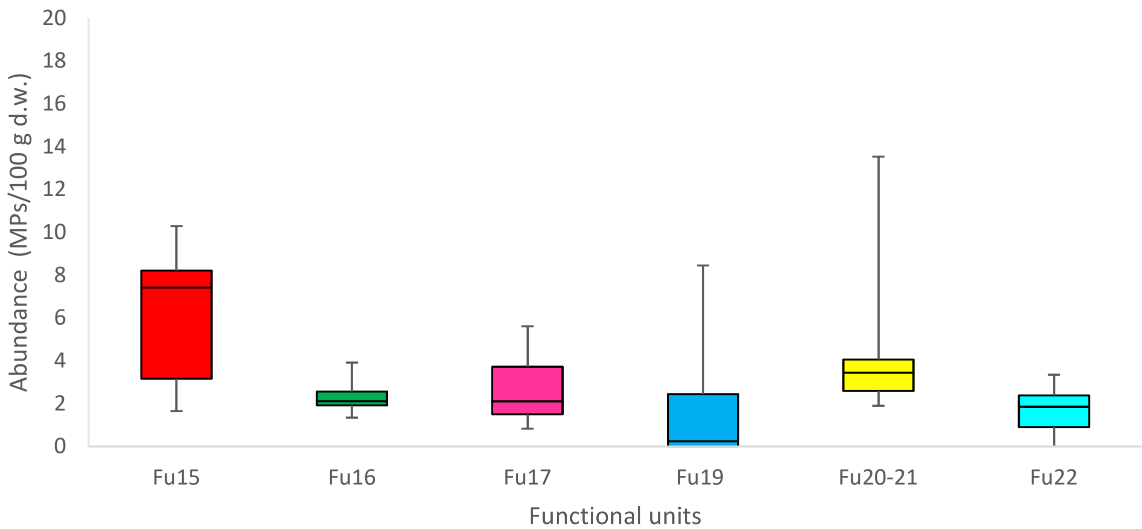


Figure 6. Boxplot showing the range in the abundance of MPs of sediments at each Functional Unit (FU 15, 16, 17, 19, 20-21 and 22), (*n*=34). Boxes represent the first and third quartiles, middle bar the median and the error bars, the maximum and minimum values.

Two main categories of MPs were recorded with the majority identified as fibres (97.1%) and the remainder fragments (2.9%). The highest average abundance of MPs was recorded at the Western Irish Sea (FU15), with the lowest abundance recorded at SE and SW Coasts of Ireland (FU19). There was a range of MP colours extracted from the sediment (*n*=104) with blue (75%) being the most prevalent, followed by red (11.5%), white (4.8%) black (3.8%), green (1.9%), and other (pink, grey and multicoloured) taking up the remaining 3%. The length of the MP fibres ranged from 126 µm to 15.269 mm. Four fibres were greater than the upper limit of 5 mm but were included in the analysis as they had a width < 20 µm. The most common size of fibres recorded was <2 mm (73%). No significant differences were observed in the level of MPs recorded in the sediments between the FU's (Kruskal-Wallis test; *p*=0.120).

A subsample (47%) of MPs were analysed for polymer identification from sediment samples across all six of the FU's. Polystyrene (PS), Polypropylene (PP) and Nylon (polyamide) (PA) were the most prevalent polymers identified in the sediment across all six of the FU's, with PS and PP found at all six sites and PA found at three.

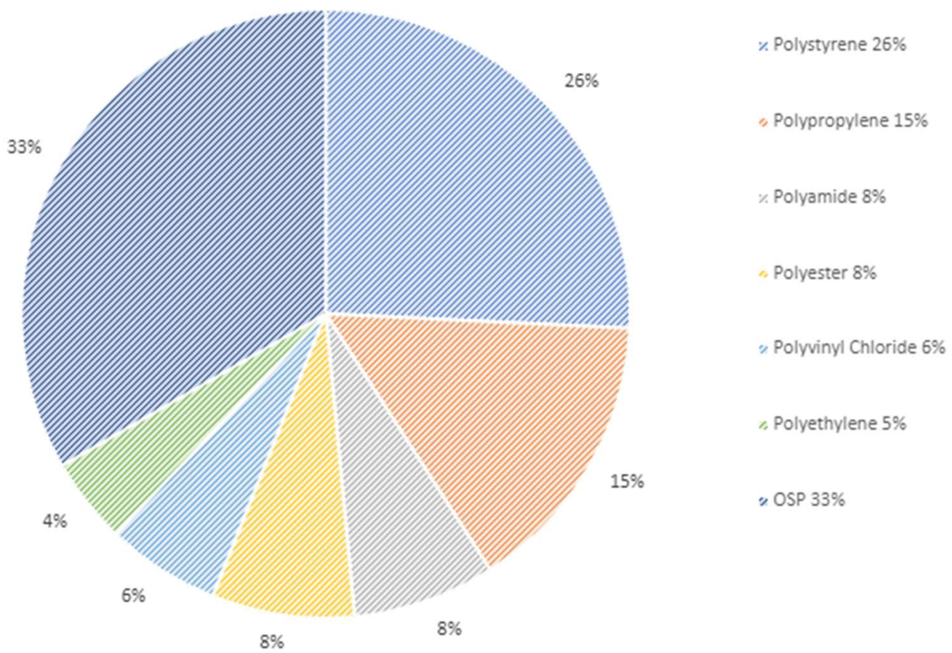


Figure 7. Polymer composition (%) of MPs found in the sediment across all six Functional Units). The main plastics are categorised by resin types according to (Plastics Europe, 2019) in conjunction with Other Synthetic Polymers (OSP).

2.4.3 Sediment characterisation

Two sediment types were identified based on their particle composition textural group (Folk, 1954): Muddy Sand (FU 19, 20-21, 22) and Sandy Mud (FU15, 16, 17). The average MP abundance in Sandy Mud was greater than Muddy Sand but no significant relationship was found between the sediment type and the abundance of MPs (Kruskal-Wallis test; $p= 0.172$). No significant relationship was found between the level of Total Organic Content (TOC) and the abundance of MPs recorded (Kruskal-Wallis test; $p=0.416$).

The relationship between MP abundance and depth was examined using a Kruskal-Wallis test, illustrating that there was no significance between MP depth and abundance ($p=0.453$). Similarly, MP abundance at distance to shore of each FU station was explored and no statistical significance was recorded (Kruskal-Wallis test; $p=0.479$). In addition, no statistically significant correlation was found between the abundance of MPs present in *N. norvegicus* and the abundances found in the sediment (Spearman's rank; $p= 0.623$).

2.5 Discussion

2.5.1 Microplastic abundance and polymer types

This study recorded slightly higher MP abundance (72%) than those previously reported in the North East Atlantic (Hara *et al.*, 2020), based on a wild population sample of 150 individuals, where 69% of samples contained MPs. Similarly, (Welden and Cowie, 2016a) recorded 67% prevalence of MPs in *N. norvegicus* populations from North and West of Scotland. A higher prevalence of MPs (83%) was reported by both (Murray and Cowie, 2011) in the Clyde Sea examining 120 individuals and by (Cau *et al.*, 2019) in the Mediterranean Sea examining 89 individuals. Another smaller study by (Cau *et al.*, 2020) recorded 100% MP abundance ($n=27$) in the Mediterranean Sea and (Martinelli *et al.*, 2021) similarly recorded 100% prevalence in 23 individuals from the Adriatic Sea.

A significant difference in MP presence in *N. norvegicus* between FU's was recorded in this study. The FU's within the Western Irish Sea (FU15) and the Smalls (FU22) showed similar high abundances and were both significantly different from all other FU's. Similarly in a recent study, the Western Irish Sea showed a higher frequency of MPs in comparison to other FU's (Hara *et al.*, 2020). Proximity to shore was not seen to be a significant factor affecting MP abundance in this study with SW and SE coasts

(FU19) having lower abundances of MPs although it was closest to shore. However, the proximity to MP sources has been recognised as a potential driver of MP ingestion by marine organisms (Franceschini *et al.*, 2021) and in this study proximity to highly industrialised coasts of Northern Ireland and Great Britain showed higher levels of MPs recorded in both sediments and *N. norvegicus* from FU15. Similarly, (Welden and Cowie, 2016a) demonstrated that nearshore habitats near anthropogenic pressures recorded a higher abundance of MPs in the gastrointestinal tract (GIT) of *N. norvegicus*. The Porcupine Bank (FU16) had the lowest abundance of MPs and was significantly different from all other FU's. This may be because FU16 is more isolated (further from shore and deeper) and is likely to be exposed to fewer anthropogenic impacts. Spatial distribution of MPs is strongly controlled by ocean currents (Hill *et al.*, 1997; Ng and Obbard, 2006; Kane Ian *et al.*, 2020) which would suggest that MP concentrations could also be diluted, potentially attributing to the lower MPs levels observed in the SW and SE coasts which are exposed to the Atlantic Ocean (FU19).

The type of MPs recovered from *N. norvegicus* and the surrounding sediment in this study were predominantly blue fibres. This finding is similar to other studies looking at benthic organisms (Welden and Cowie, 2016a; Wang *et al.*, 2019; Hara *et al*., 2020; Fang *et al.*, 2021) and in sediments in Irish waters (Martin *et al.*, 2017; Pagter *et al.*, 2020b). This highlights the likelihood of fibres as the most abundant type of MP readily available in the marine environment (Wright *et al*., 2013; Rebelein *et al.*, 2021). The results are similar to other studies which found blue to be the most common MP colour recorded (Hara *et al.,* 2020; Pagter *et al.*, 2020a; Zhang *et al.*, 2020a). Entangled balls of fibres have been previously recorded in the GIT of *N. norvegicus* (Murray and Cowie, 2011; Welden and Cowie, 2016a), however, they were not a prominent feature in this study.

The importance of using a secondary form of MP identification such as a FT-IR Microscope was highlighted by Pagter *et al.,* (2020a) as approximately 20% of the MPs retrieved were identified as natural. The current study identified 5% of the MP subsamples from *N. norvegicus* as natural fibres. The most common polymer of plastic found to be present in *N. norvegicus* was Polystyrene (PS), which is used for food packaging, electrical and electronic equipment, building insulation, inner liner for fridges etc. (Plastics Europe, 2019); this correlates with findings from (Hara *et al*., 2020). The next two most common polymers were Polypropylene (PP) which is used for food packaging, sweet wrappers, microwave containers, pipes, bank notes, etc. (Plastics Europe, 2019) and Nylon which is used for fishing nets and ropes (OSPAR COMMISION, 2020). It is notable that all three polymers are used in the fishing and aquaculture industry (EUNOMIA, 2018). Both PP and PA have previously been identified as the most frequently observed polymers in the GIT of *N. norvegicus* in the North and West of Scotland (Welden and Cowie, 2016a). Plastics with a higher density than water are expected to have increased settling rates in comparison to lower density plastics (Schwarz *et al.*, 2019). In this study both higher density MPs (PS and PA) and lower density MPs (PP), were retrieved from benthic sediment. This may be due to microbial growth on pieces of plastic (biofilm) which can alter their density causing them to sink (Semcesen and Wells, 2021) and/or fragmentation of fishing gear already present in the environment which is known to shed MPs (Saturno *et al.*, 2020; Napper *et al.*, 2022).

It has been acknowledged that contaminant levels in organisms may be closely related to the levels found in the surrounding environment (Qu *et al.*, 2018). To date, there are a lack of integrated studies investigating MPs in marine organisms and their surrounding environment; thus the relationship between them still remains unclear (Qu *et al.*, 2018). In a study carried out in the coastal waters of China, a positive relationship between MP levels in two species of mussels and in the surrounding waters was established for not only the abundance of MPs but also for MP characteristics (Qu *et al.*, 2018). In this study, the most common polymers found in the sediment were Polystyrene (PS), Nylon (PA) and Polypropylene (PP) which mirror the MPs found in the GIT of *N. norvegicus,* highlighting the potential, if not probable link between environmental prevalence and MP abundance in organisms. A study looking at MPs in bivalves had similar findings to this study, where MPs present in the organisms had the same characteristics as those found in the surrounding seawater although no significant relationship was observed (Cho *et al.*, 2021).

2.5.2 Microplastic abundance and characteristics of *N. norvegicus*

Both Murray and Cowie (2011); Welden and Cowie (2016a) suggested that MPs are excreted through ecdysis (moulting process) and assume this to be a key route of excreting MPs aggregations. Furthermore, Welden and Cowie (2016a) recorded lower levels of MPs in the stomachs of individuals that had recently moulted and identified fibres in the discarded gut lining of moulted individuals. The results of this study, however, are in line with (Hara *et al*., 2020), where no significant association was found between MP abundance and moult stages. It has also been acknowledged that the abundance of plastic cannot be directly linked to a single factor due to many confounding variables (Lusher *et al*., 2017; Vendel *et al.*, 2017).

Crustaceans have a complex digestive tract in comparison to other invertebrates (Welden and Cowie, 2016a) with the presence of chitinous plates in the foregut (Murray and Cowie, 2011). The shape of the plates narrows at the entrance to the hindgut, which may prevent MPs from being egested. Research has suggested that as the organism grows, the gaps within the gastric mill also increase (Welden *et al.,* 2015) therefore, allowing for the possibility of larger individuals to egest more MPs in comparison to smaller individuals (Welden and Cowie, 2016a). The current study shows *N. norvegicus* of less than 82 mm TL are seen to have a higher average MP abundance than larger individuals >127 mm TL, which are in alignment with findings by (Murray and Cowie, 2011; Welden and Cowie, 2016a) where *N. norvegicus* containing higher abundances of plastics in their stomachs had smaller carapace lengths (CL). However, each FU revealed variation between MP abundance and TL of organisms with contradictory findings further highlighting the ubiquity and the heterogeneity of MPs observed in the marine environment. The smallest organisms were recorded in the Western Irish Sea (FU15; TL of 87.4 ± 13.5 mm) in comparison to the larger *N. norvegicus* found in the Porcupine Bank (FU16; TL of 104.1 ± 16.3 mm). This aligns with current stock assessments where high burrow densities in FU15 are associated with relatively smaller organisms relative to the larger organisms and low burrow densities observed in FU16 (Johnson *et al*., 2013; Lundy *et al.*, 2019; Aristegui *et al.*, 2020). Furthermore, smaller organisms were recorded to have higher MP abundance in comparison to larger organism's, contradictory to findings by Hara (2020) which suggested that the highest abundance of MPs were in larger organisms. Welden and Cowie (2016a) stated that the abundance of MP may be reflective of the discrepancy in size and moulting frequency between males and females. Female *N. norvegicus* moult at a slower rate in comparison to males, and therefore, may have a smaller gastric mill making the egestion of MP particles more difficult and possibly retained for a longer period (Welden and Cowie, 2016a). Females have been thought to retain plastic for twice as long as males due to the reduced moult rate and resulting smaller size (Welden and Cowie, 2016a). Similarly, and in agreement, in this study females had the highest abundance of MPs; however, a significant difference was only illustrated within two FU's (FU15 and FU22). Despite this the authors remain cautious, as the results obtained in this study (variation in MP abundance as a function of sex and TL, and as a result of gastric mill size, and or moulting) all showed low overall MP abundance. Such low values increase potential for false positive/negative results, or indication of no relationship.

The dinoflagellate parasite *Hematodinium* spp. infects commercially valuable crustaceans such as *N. norvegicus* (Li *et al.*, 2021; Stentiford and Shields, 2005), with the parasite having been previously recorded in Irish waters (Briggs and McAliskey, 2002). However, no relationship between the infected organisms and MP abundance was established in this study. The authors are aware of the limitations of the detection methods used, with the colour method having been shown to detect 50% less infections in comparison to the pleopod method (Stentiford *et al.*, 2001). The pleopod method, however, can only detect heavily infected individuals (Small *et al.*, 2006) and is open to subjectivity (Stentiford *et al.*, 2001). Therefore, the potential of infection rates is likely to be underestimated in this study.

Feeding behaviour and the prevalence of MPs in the surrounding environment are two of the main factors that can influence MP abundance in organisms (Murphy *et al.*, 2017; Walkinshaw *et al.*, 2020). A direct relationship between MP abundance in *N. norvegicus* and the surrounding environment has not been established to date (Murray and Cowie, 2011; Martinelli *et al.*, 2021). However, diets of *N. norvegicus* have been found to mirror local food availability (Parslow-Williams *et al.*, 2002), with a recent study suggesting a possible relationship between proximity to macroplastic hotspots and MPs in benthic organisms using a generalised additive model (GAM) (Franceschini *et al.*, 2021). The results of the current study indicate that MPs do not accumulate, as larger organisms, who are older, had lower MP abundances. A recent study where separate stomach and intestines examinations of *N. norvegicus* were conducted revealed higher abundances of MPs in the intestine, suggesting their ability to move through the GIT, eventually being excreted (Cau *et al.*, 2020). This is in alignment with the results of our current study.

The prevalence of similar types, colours, abundances, and proportional compositions of MPs in the GITs of *N. norvegicus* and in their habitat sediments empirically indicates MP deposition to the seafloor, through either direct sinking from the water column to the sea floor or through intermediary consumers, acts as a pathway to ingestion by *N. norvegicus*. The vertical transportation of MPs to the deep-sea is complex and poorly understood (Courtene-Jones *et al.*, 2017; Barrett *et al.*, 2020) with the sinking rates of plastic particles influenced by many factors including particle size, shape and polymer density (Zhang, 2017; Kooi *et al.*, 2018). While this study may not explicitly substantiate this, it is a hard to conclude otherwise, and is in line with potential pathways suggested by Coyle *et al.*, (2020). It may be hypothesised that the explicit amounts of MPs present in the GIT of *N. norvegicus* relate to complex interactions of individual anatomy, larger North Atlantic and localised oceanographic conditions, and environmental availability including proximity to point and diffuse sources and biotic and abiotic pathways to sediment and the food chain.

2.5.3 Microplastics, food security and consumer confidence

Seafood is an important part of healthy diets across Europe (FAO, 2020) with the total seafood production in 2018 amounting to 179 million tonnes and this is expected to further rise to 204 million tonnes by 2030 (FAO, 2020). Therefore, assessing potential human consumption of MPs from seafood is imperative, as a potential exposure pathway (Wright and Kelly, 2017; Smith *et al.*, 2018; De-la-Torre, 2020). Furthermore, food security may be negatively impacted by the presence of MPs in seafood, however, data and evidence pointing to the existence of a relationship between MPs and food security is still lacking (De-la-Torre, 2020; Walkinshaw *et al.*, 2020).

Recent studies have identified MPs in human stools (Harvey and Watts, 2018; Zhang *et al.*, 2021) indicative that humans can pass MPs. Reviews have identified that exposure can occur through skin contact, inhalation or ingestion (De-la-Torre, 2020). Although there have been more studies on ingestion, it has been hypothesised that humans are more exposed to microplastics *via* inhalation rather than ingestion (Vianello *et al.*, 2019; Zhang *et al.*, 2020b). The true effects of exposure are, however, still unknown (Barboza *et al.*, 2018; Vethaak and Legler, 2021). A lower MP risk of human ingestion is evident in organisms where the GIT is discarded prior to human consumption (Murray and Cowie, 2011; Wright and Kelly, 2017). Furthermore, it must be noted that humans are not only exposed to MPs through seafood consumption but also from other sources such as drinking water (Oßmann *et al.*, 2018), tea bags (Hernandez *et al.*, 2019), beer (Liebezeit and Liebezeit, 2014), honey (Liebezeit and Liebezeit, 2013) airborne particles (Bergmann *et al.*, 2019) and dust (Gallagher *et al.*, 2015). MPs have recently been identified in the edible tissue of *N. norvegicus,* highlighting the possibility of translocation of small plastic particles, however, prudence must be taken when interpreting such results (Martinelli *et al.*, 2021). Mechanisms of translocation of small microplastics remain unclear (Wang *et al.*, 2016) and further studies need to be conducted to assess microplastics in commercially relevant species as well as providing consumer confidence. In comparison to other regions in the Mediterranean, MP abundances in *N. norvegicus* in the North East Atlantic are relatively low (Cau *et al.*, 2019; Martinelli *et al.*, 2021), hence, exposure by human consumption is also considered low (Hara *et al.,* 2020). Of note is the prevalence in this study of MP fibres over fragments, while Martinelli *et al.*, (2021) reported a predominance or fragments to fibres at a ratio of 3:1. Further, it may be speculated that the much higher abundaces reported by Martinelli *et al.*, (2021) are a result of samples being from the relatively encolsed Adriatic Sea in the Mediterranean basin in comparison samples from the North East Atlantic, Celtic and Irish seas analysed here.

2.5.4 Monitoring

Given that the marine environment is everchanging, concentrations of MPs are known to fluctuate over time (Hill *et al.*, 1997; Frias *et al.*, 2020), and although the current risk to humans is low, it is still of paramount importance to monitor plastic contamination to ensure MPs levels remain low. *N. norvegicus* have been previously proposed as an indicator species for plastic pollution (Cau *et al.*, 2019; Welden, 2015; Franceschini *et al.*, 2021). Even though this study does not allow for a distinct relationship to be identified it does demonstrate that *N. norvegicus* may be used as a bioindicator for marine MP pollution, as it meets the selection criteria and reflects the concentration of contaminants in its surrounding environment (Markert *et al.*, 2003; Fossi *et al.*, 2018). For example, *N. norvegicus* are benthic opportunistic feeders of high commercial importance and are widely distributed around the North East Atlantic and the Mediterranean (Ungfors *et al.*, 2013; Cau *et al.*, 2019; Hara *et al.,* 2020). Furthermore, in the Mediterranean, *N. norvegicus* has already been suggested as a bioindicator for MP presence on the seafloor for small – scale (FAO Geographical subareas, GSAs) (Fossi *et al.*, 2018).

It is important to note that a single species approach may not give a complete overview of MP prevalence in the environment, as it only represents a snapshot of what that organism has recently consumed, while organisms from different FU's, guilds (groups of species exploiting a comparable series of resources) and trophic levels could present different levels of MPs available in the environment (Pagter *et al.*, 2020a). This suggests that the limitations of a single species indicator need to be acknowledged, and preferable further investigated, if implemented in a monitoring programme or that a more ecosystem-wide approach should be applied, and clearly so where a suitable single species bioindicator is not available (Pagter *et al.*, 2021).

2.6 Conclusion

This study provides baseline data on the occurrence of MPs in *N. norvegicus* and its surrounding environment in the North East Atlantic Ocean. It is apparent from the results that *N. norvegicus* in the Porcupine Bank FU16 had substantially lower MP abundance in comparison to the other sites and the Western Irish Sea FU15 had the highest abundance. Importantly, results imply that MPs do not bioaccumulate in *N. norvegicus* and the size of organism may have an influence on MP abundance with the larger *N. norvegicus* having lower MPs, however, there is no individual biological parameter that correlates with the MP abundance recorded.

While research suggests an ecosystem-based approach is the most reliable, *N. norvegicus* in combination with the sediment does provide potential as a monitoring tool but limited to the presence or absence of microplastics in an area and could potential be the basis of a new traffic light system to reflect the levels of bioavailable microplastics. Further research is required to develop this system to define categories of MP abundance.

When assessing the bioavailability of MPs in the environment it may be hypothesised that an ecosystem-based approach, reporting MP loadings in a number of pathways should be applied in order to collect environmentally relevant data to fully inform the MSFD (Descriptor 10 - Marine Litter). This study demonstrated MP presence in *N. norvegicus* and associated substrata, and complexities in ascertaining a robust contaminant level relationship. *N. norvegicus* may form a readily available bioindicator for marine MPs, however further investigation into the MP abundance in *N. norvegicus* and its surrounding environment is recommended to better establish MP retention, mechanisms, patterns, and hotspots.

Acknowledgements

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3. Size dependent egestion of polyester fibres in the Dublin Bay Prawn (*Nephrops norvegicus*)

This chapter is a verbatim reproduction of the following manuscript:

Joyce, H., Nash, R., Kavanagh, F., Power, T., White, J., Frias, J, 2022. Size dependent egestion of polyester fibres in the Dublin Bay Prawn (*Nephrops norvegicus*). Marine Pollution Bulletin, 113768. DOI: https://doi.org/10.1016/j.marpolbul.2022.113768

Research Highlights:

- Retention and egestion times of different length polyester fibres are size-dependent for *Nephrops norvegicus.*
- Microplastic fibres of 3 mm in length were excreted within 24 hours.
- Microplastic fibres of 5 mm in length were retained for a minimum 72 hours.
- Microplastic fibres of 10 mm in length were retained for a minimum 96 hours.
- Fibres of 5 and 10 mm were observed in entangled balls of unintroduced fibres, potentially increasing their retention time.

Keywords

Microplastic; Egestion; Pollution; *Nephrops norvegicus*; Retention.

3.1 Abstract

Microplastics (MPs) are an extensive global contaminant in the marine environment, known to be ingested by marine organisms. The presence of MPs in the commercially important marine decapod crustacean *Nephrops norvegicus* (Dublin Bay Prawn) has been documented for the North-East Atlantic and the Mediterranean, however, uncertainties remain about retention times of MPs in the

gastrointestinal tract (GIT) of this species. This study aims to investigate the retention times of polyester MP fibres of three sizes (3, 5, and 10 mm in length) and to determine whether the egestion of MP fibres is size and time dependent. Results suggest that MP fibres of different lengths are retained for different periods of time, with larger MP fibres being retained for longer periods (e.g., minimum 96 hours for 10 mm fibres). The present study also assesses for the first time, the size dependent relationship of MP fibres under controlled conditions for *N. norvegicus*.

3.2 Introduction

Global plastic production reached almost 370 million tonnes in 2019, with Europe's production alone contributing 58 million tonnes (Plastics Europe, 2020). The ever-increasing use of plastic in society has led to MPs accumulation in the natural environment (Ostle *et al*., 2019). Plastic marine litter and microplastic (MP) pollution in the ocean originates essentially from land (Jambeck *et al*., 2015, UNEP, 2021). Consequently, it has been estimated that over 690 species have had interactions with marine debris, particularly through entanglement (Gall and Thompson, 2015), and ingestion, with over 300 species reported to directly ingest MPs (Kühn *et al*., 2015). MPs are known to affect marine organisms at all trophic levels, from zooplankton (Md Amin *et al*., 2020) to marine mammals (Nelms *et al*., 2019). MPs, defined as synthetic solid plastic particles or polymeric matrices, which have a size of less than 5 mm in length and result from both primary or secondary origin (Frias and Nash, 2019) are a major threat due to their persistent nature in our oceans (Jambeck *et al*., 2015).

MP ingestion by marine biota is a reason for concern, particularly for organisms at lower trophic levels which have been seen to have higher MP contamination abundances (Walkinshaw *et al*., 2020). Studies on MP ingestion often report varying results however, for example, an exposure trial of MPs in the marine Isopod, *Idotea emarginata,* showed no negative effects from ingestion of particles (Hämer *et al*., 2014). Similar findings were observed by Kaposi *et al*., (2014) on MP ingestion by the Sea Urchin, *Tripneustes gratilla*, whereas the lugworm (*Arenicola marina*) was observed to have significant weight loss (Besseling *et al*., 2013). Moreover, the pacific mole crab, *Emerita analoga,*a decapod crustacean, showed a higher level of mortality in crabs exposed to MPs than to the non-MP exposed control organisms (Horn *et al*., 2020).

The residence time of MPs within an organism is termed "retention time" and plays a pivotal role in understanding their effects (Yu *et al*., 2021). There have been several factors attributed to the varying retention times of MPs in marine organisms including species (Roch *et al*., 2021), presence of food (Bour *et al*., 2020), MP size (Brillant and MacDonald, 2000), MP type (Bour *et al*., 2020), MP concentration, and MP exposure time (Lu *et al*., 2016, Hu *et al*., 2022). The retention time of MPs in organisms varies greatly depending on species: e.g., Fathead Minnows, *Pimephale promelas,* retained

MPs for 12h (Hoang and Felix-Kim, 2020), while the Brine Shrimps, *Artemia,* ranged between 2 and 72h (Bour *et al*., 2020) and the Shore Crab, *Carcinus maenas,* recorded much longer retention times of between 14 and 21 days (Watts *et al*., 2015). A longer MPs retention time may cause adverse effects on an organism, including internal blockages, injuries, inflammation and even toxicity (Welden and Cowie, 2016a, Yu *et al*., 2021). It has also been suggested that MP fibres are more toxic than beads (Au *et al*., 2015, Gray and Weinstein, 2017), showing that MP shape might play a role when ingested. However, it has been documented that many organisms have the ability to excrete MP fibres once ingested (Rebelein *et al*., 2021).

Laboratory feeding experiments under a controlled environment play a significant role in understanding the effects of MP exposure to marine biota through ingestion (Murray and Cowie, 2011, Watts *et al*. 2014, Welden and Cowie, 2016b, Hankins *et al*., 2018, Rebelein *et al*., 2021). Laboratory assays targeting size-dependent exposure show variation between species and MP sizes (Welden and Cowie, 2016b, Gray and Weinstein, 2017, Kinjo *et al*., 2019) with most of the size-dependent exposure studies using nano- and/or MP particles <100 µm (Jeong *et al*., 2017, Rist *et al*., 2017, Kinjo *et al*., 2019). The upper size range of MPs chosen for laboratory experiments is largely dependent on the size of the organism and its diet (Yu *et al*., 2021).

MPs have been previously identified in the gastrointestinal tract (GIT) of many organisms including the decapod crustacean, *Nephrops norvegicus* (Welden and Cowie, 2016a, Cau *et al*., 2019, Cau *et al*., 2020, Hara *et al*., 2020, Joyce *et al*., 2022), however, limited studies on retention time have been conducted (Murray and Cowie, 2011, Welden and Cowie, 2016b). *N. norvegicus,* commonly referred to as the Dublin Bay Prawn or the Norway Lobster is considered a particularly important commercial species and is considered a delicacy food product in Europe (Ungfors *et al*., 2013). In 2019, a total of 8,100 tonnes of *N. norvegicus* were landed by the Irish fleet with a total value of €59 million (BIM, 2019).

Found in muddy marine benthic environments (Welden *et al*., 2015; Cau *et al*., 2020) *N. norvegicus* are opportunistic scavengers with a diet composed of molluscs, echinoderms and crustaceans (Murray and Cowie, 2011, Welden *et al*., 2015) with non-food materials (inert objects described as stones and synthetic fibres), also recorded in their stomachs (Parslow-Williams *et al*., 2002). This non-selective feeding behaviour is a probable reason for MP ingestion by these organisms (Cau *et al*., 2019, Hara *et al*., 2020).

Although MP fibres released from textiles are a recognised source of marine plastic pollution (Napper and Thompson, 2016, De Falco *et al*., 2018), most laboratory studies on the effects of MPs focus mainly on polystyrene microspheres (Cong *et al*., 2019, Hoang and Felix-Kim, 2020, Eom *et al*., 2021, Yu *et al*., 2021), while little information is available on MP fibres of other materials. Furthermore, physical characteristics, such as the size of marine plastic litter particles, are a pivotal measure in monitoring owing to the potential size dependent effects on organisms (Kershaw *et al*., 2019; Franceschini *et al*., 2021). This study investigates MP retention times of MP polyester fibres in live *N. norvegicus* and determines whether egestion of fibres is size-dependent in a short-term exposure trial.

3.3 Materials and Methods

3.3.1 Collection/Sampling of *Nephrops norvegicus*

Live *N. norvegicus* samples were creel caught in mid-spring from Clew Bay (53° 49' 58.897''N; 9°45'58.717'' W) (Fig. 1), a west-facing bay that contains an archipelago of small islands and interlocking bays, on the west coast of Ireland (Keaveney *et al*., 2006). The individuals collected were representative of a commercial catch.

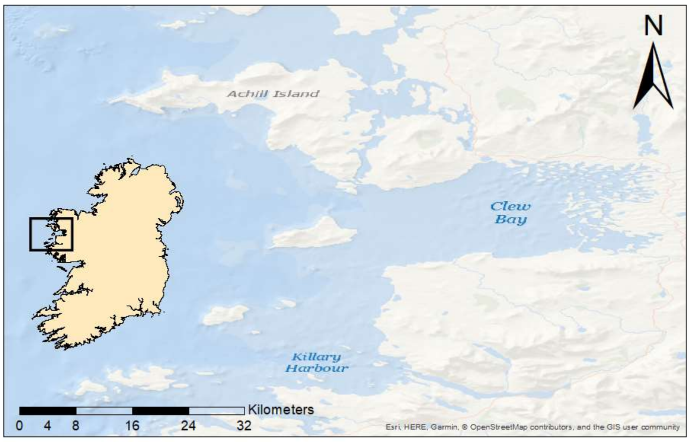

Figure 1. *N. norvegicus* sampling site on Clew Bay, Bellmullet, Co. Mayo.

The *N. norvegicus* samples used in the experiment were transported to the Marine and Freshwater Research Centre (MFRC) at the Galway-Mayo Institute of Technology (GMIT) and were placed into individual compartments in holding tanks, with recirculating seawater systems housed in a constant temperature (CT) room. The recirculating system compromised of a tropical marine TM2500 water treatment system, with mechanical, sand, and biological filters, a trickle tower to regulate dissolved gasses and its own chiller. Water temperature, salinity and photoperiod were kept at 10 ± 1 °C, 35‰ with an 8-hour light and 16-hour dark cycle, to simulate natural habitat conditions. Organisms were left to depurate and acclimatise for a one-week period. During the acclimatisation period organisms were assessed for healthy behaviour (i.e., carrying out sweeping movements with antennae as they explore their environment (Krång and Rosenqvist, 2006)), and only those that displayed healthy conditions were chosen for the experiment.

After the acclimatization period in the holding tanks, the carapace length, total length, weight, sex, physical damage, carapace condition and moult stage were recorded. The sex was determined by the structure of their sexual pleopods (Farmer, 1974). The physical damage of each organism was assessed by observing the external structure, based on a damage index proposed by Ridgway *et al*., (2006) which categorises the structural damaged caused to the specimen on claws, limbs, eyes, and soft tissue into three categories (a) no damage, (b) lightly damaged, and (c) heavily damaged. The carapace condition and moult stage were determined by following the methodology by Milligan *et al*., (2009).

Individuals were randomly selected and placed into 50 L tanks, which were divided into two sections with equal areas, and were left to acclimatise for at least 48 hours. Based on initial behavioural observations from our baseline analysis *N. norvegicus* were placed individually in separate tank sections for the microplastic feeding experiment, and furthermore to avoid social stress such as cannibalism (Devriese *et al*., 2017). The experimental tanks were filled with aerated synthetic seawater prepared for the experiment using Red Sea © "Coral Pro Salt". The tanks were continuously aerated, and temperature, pH and ammonia were monitored daily.

3.3.2 Egestion rates and behaviour

In a baseline analysis on food egestion rates and behaviour analysis of *N. norvegicus*, a dozen organisms were fed MP-free shrimp, which were dyed with Goodall's red food colouring so that it could be tracked. The egestion rate of food and behaviour of the organisms were monitored every half an hour for a time series of 10 hours, and then again at 24 hours. Times of initial feeding, food consumption and egestion were recorded to assess egestion rates. The behaviour of organisms throughout the experimental period was also recorded to ensure healthy behaviour.

3.3.3. Fibres and feed

Outside of the baseline the organisms (*n*=46) were divided into three experimental groups (Figure 2). Shrimp was used as the primary food source for *N. norvegicus* based on previous laboratory experiments (Cristo, 1998, Cristo, 2001). Each group was fed 1 g of fresh shrimp (*Pandalus borealis*) caught by trawls in the North Atlantic. Fibres were the chosen form of MP in this study, as they are the most common MPs recorded in the marine environment previously recorded in *N. norvegicus* (Rebelein *et al*., 2021, Joyce *et al*., 2022). In addition, MP beads were trialed, however, the dyed MP beads were easily dislodged from the food into the water during the feeding activity of *N. norvegicus.* These beads were identified floating in the tank using an UV fluorescent torch (Vansky ZQ-X1119B). Therefore, Taklon fibres, a smooth and soft polyester derivative, obtained from a commercial makeup brush were the only MP seeded into food. Fibres were cut to set lengths of 3 mm, 5 mm and 10 mm and stained using Nile Red (75mg of Nile Red stock solution with 75ml of acetone (CH_3COCH_3)) making them easily identifiable, following (Maes *et al*., 2017, Hara *et al*., 2020). The set lengths of fibres were chosen to represent the food size *N. norvegicus* usually ingests (Thomas and Davidson, 1962). Fibres in Nile red solution were vortexed for 1 minute and allowed to rest overnight in the fume hood until the remaining acetone solution evaporated. Fibres were then rinsed with ultra-pure water to remove excess solution. These fibres are easily identifiable because they glow under ultraviolet light. Control organisms were fed MP-free shrimp.

3.3.4 Microplastic exposure Trial

The retention experiment took place over three separate trials (Figure 2). Each trial included control organisms (*n*=4), where the initial two were analysed at the beginning of the experiment and the remaining two analysed on the last day of the trial. Freshly caught *N. norvegicus* were used for each trial. The first trial had *n*=16 treatment organisms which were fed MP seeded food containing five 3 mm fibres. The second trial had *n*=8 treatment organisms which were fed food seeded with five 5 mm fibres and the third trial had *n*=10 treatment organisms that were fed food seeded with five 10 mm fibres. After feeding took place, in each trial two organisms were humanely euthanized at t0 and every 24hrs after that. Organisms were euthanized using a clove oil with a concentration of 900 µL/L which is mainly made up of 80-95% eugenol and has been used previously on *N. norvegicus* (Cowing *et al*., 2015) as a humane method of euthanasia under laboratory conditions (Gardner, 1997, Wong, 2013). Organisms were then stored in a freezer and defrosted prior to dissection to determine MP retention. To account for all the introduced MP fibres, any faeces and/or left-over pieces of food were examined, while the synthetic seawater in the experimental tanks was filtered using a vacuum pump (VCP130) through a 47mm Whatman® (GF/C – 1.2 µm pore size) glass microfiber filter paper after the experiment.

Trial		t0 control	t0	t24	t48	t72	t96	t120	t144	t168	Last day control
Trial 1 (3mm)	Number of Organisms	2	2	2	2	2	2	2	2	2	2
	Number of fibres per individual	0	5	5	5	5	5	5	5	5	0
Trial 2 (5mm)	Number of Organisms	2	2	2	2	2	-	-	-	-	2
	Number of fibres per individual	0	5	5	5	5	-	-	-	-	0
Trial 3 (10mm)	Number of Organisms	2	2	2	2	2	2	-	-	-	2
	Number of fibres per individual	0	5	5	5	5	5	-	-	-	0

Figure 2. Experimental design of the exposure trials showing the number of organisms and MPs per individual at each sampling slot.

3.3.5 Microplastic analysis in N. norvegicus

MP analysis was carried out on two separate portions of the GIT (the stomach and the intestine), as previously suggested by Cau *et al*., (2020). Once removed, these organs were immediately transferred to decontaminated labelled jars. Digestion was carried out using a 10% potassium hydroxide (KOH) solution at 40°C for 48 hours, as recommended by Hara *et al*., (2020). The digestive solution was filtered using a vacuum pump (VCP130) through 47mm Whatman® (GF/C – 1.2 µm pore size) glass microfiber filter paper. The filter was then transferred onto a labelled petri dish for visual examination and identification of the introduced dyed MP fibres.

3.3.6 Contamination control

Cross-contamination was reduced by using a 100% cotton lab coat (Pagter et al., 2018) and the wearing of synthetic clothing was avoided (Hermsen et al., 2018). Decontamination of glassware jars was carried out using dilute Nitric Acid Bath (10%). All surfaces and dissection equipment were cleaned before and after the dissection of each organism to avoid cross contamination. Air controls were used during dissections and filtering. Blanks were run using ultra-pure water, KOH (10%) and feed was digested and analysed to ensure sourced food did not contain fibres.

3.4 Results

Of the *N. norvegicus* examined for the MP exposure trial (*n*=46) the majority were determined to be male (80.4%) and the remaining 19.6% identified as female. All individuals were sexually mature, falling within the estimated Carapace length (CL) range of 23.2 to 27.6 mm for females and 25.9 to 31 mm CL for males, as reported by McQuaid *et al.,* (2006). CL ranged between 37 and 56mm, with a mean of 47.76 ± 3.92mm. The organisms had a mean total length (TL) of 120.2 ± 18.7mm and a mean mass of 49.8 ± 14.4g. Most individuals (89.1%) were observed to have a hard carapace condition, which is assumed to be at the intermoult stage. Organisms with a soft carapace condition represented 10.9% of the sample, and assumed to be at late intermoult, or recent moult stage. No organisms at the "jelly" moult stage were used in the study.

3.4.1 Egestion rates and behaviour

The baseline analysis of food egestion saw all organisms that consumed food, egest the food between 6 and 24 hrs. Subsequently, the first sampling time in the MP feeding experiment was based on this 24 hr. Faeces were easily identified due to red dye. *N. norvegicus* was recorded every half an hour, in a laboratory setting, to assess acclimatization through activity, response to food and overall behaviour. During the experiment, individuals displayed a range of behaviour including, for example, fighting between organisms over food resources, cheliped pushing, wrestling, exploring the tank, reacting to food, and carrying out tail flips.

3.4.2 Microplastic exposure trial

Organisms introduced to experimental tanks acclimatised well, with all individuals observed to display similar healthy behaviours to those in the baseline analysis. During the experiment, *N. norvegicus* displayed similar behaviour such as actively exploring their new surroundings. Individuals were seen to react when food was introduced by either initially displaying defensive behaviour ready to fight (where individuals stood high on legs and horizontally spread chelipeds) and/or antennule "flicking" behaviour, before approaching the food and transferring it the maxillipeds and passing to the mouth.

All individuals were observed while feeding to confirm that MP fibre seeded food was ingested. However, no organisms were observed to ingest MP beads. *N. norvegicus* actively break up food while eating, therefore it is not possible to ascertain what proportion of MP fibres were ingested. In Trial 1 (3 mm fibres), treatment organisms at the initial sampling time (t0) had introduced MP fibres present in their stomachs. No MPs were identified in the treatment organisms between t24 and t168. In Trial 2 (5 mm fibres), MPs were identified in the stomachs of *N. norvegicus* at t0, t48 and t72, however, no fibres were identified in organisms at t24. Here fibres were recorded from the uneaten shrimp and the water. In Trial 3 (10 mm fibres), all 10 of the organisms that were fed plastic seeded shrimp had introduced plastics in their stomachs across all sampling times (t0 to t96) (Figure 3). The fibres not accounted for in the stomachs of *N. norvegicus* were retrieved from the filtered water in the experimental tanks.

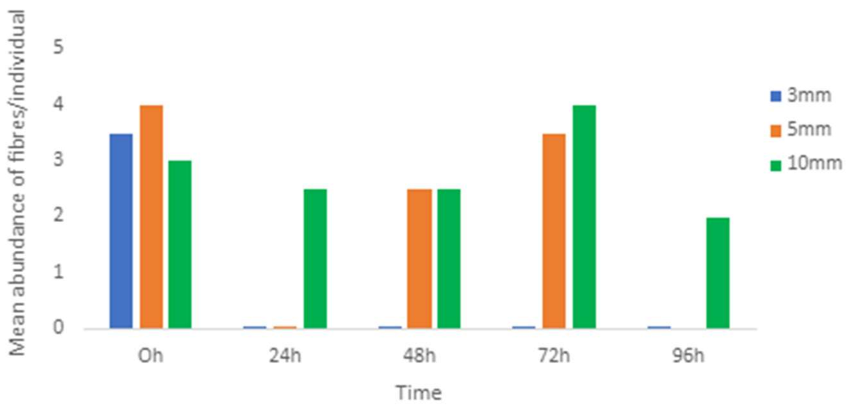

Figure 3. Mean MP retention in *N. norvegicus* of polyester fibres of three sizes (3, 5, and 10 mm).

Fibres in the form of entangled balls were identified in the stomachs of six individuals from the three trials. At least one introduced red fibre can be clearly seen to penetrate the entanglement of fibres in the stomach, in three of these individuals (Figure 4). Control organisms from all trials contained no introduced MPs at either t0 or the final time within the experimental Trials (t72, t96, t168). Introduced fibres were only identified in the stomachs. No introduced MP fibres were found further down the digestive tract in the intestines of any of the treatment organisms. Analysis of the stomach contents of both control and treatment organisms for all Trials revealed MPs to be present which were not introduced through laboratory feeding.

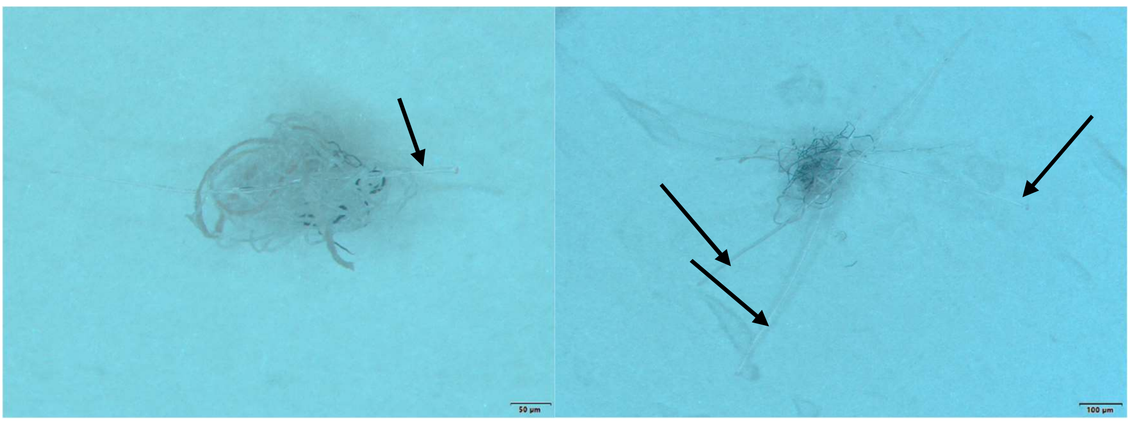

Figure 4. Two entangled balls of fibres extracted from *Nephrops norvegicus, where an introduced MP from feeding (highlighted with an arrow).* Left: 5mm fibre recovered at t72 (trial 2) from the foregut of a female. Right: 10mm fibres (n=3) recovered at t72 (trial 3) from the foregut of a male.

3.5 Discussion

This is the first study, to the authors knowledge, to investigate MP fibre retention time and associated behaviour in *Nephrops norvegicus.* Previous studies have recorded similar *N. norvegicus* behaviour, under both laboratory conditions and in the natural environment (Rice and Chapman, 1971, Newland and Chapman, 1989, Krång and Rosenqvist, 2006, Katoh *et al*., 2008), confirming organisms were not displaying any signs of stress or unnatural behaviour during the experimental assay.

Feeding studies have reported, the GIT of *N. norvegicus* to be almost completely empty 12 hours after food ingestion (Sardà and Valladares, 1990). To ensure sufficient time for organisms to egest seeded food, a sampling interval of 24 hr was chosen. A previous short-term feeding experiment of 24 hours, using 5 mm plastic fibres carried out on this species, found that the introduced fibres were present in stomachs after this ingestion period (Murray and Cowie, 2011), suggesting that MP fibres are not egested at the same rate as food items. Uncertainties remain over the retention time of MPs in the GIT and other physico-chemical behaviour of the ingested particles themselves (Roch *et al*., 2021).

On the introduction of food during all trials, individuals either displayed defensive behaviour and/or antennule "flicking" prior to approaching and transferring to the mouth. These behaviours have been also recorded by Krång and Rosenqvist, (2006) during feeding, and by Katoh *et al*., (2008) in a trial on fighting behaviour.

Preliminary assay trials with MP beads demonstrated that the ease with which they became dislodged and released into the surrounding water during the feeding activity of *N. norvegicus,* led to the beads being disregarded from further trials. In a previous short-term exposure trial by Devriese *et al*., (2017), ingestion of microbeads (6-600 µm) by *N. norvegicus* were seen to have no impact on their nutritional state; however, a long-term retention study using fibres (3-5 mm) revealed a decrease in the nutritional state and false satiation, possibly due to the retention of fibres in the foregut (Welden and Cowie, 2016b). This shows the potential ability of beads to be easily egested due to their round shape versus irregular shaped MPs such as fibres and fragments, which may be retained for longer periods (Yu *et al*., 2021). MP beads are not commonly found in *N. norvegicus* (Cau *et al*., 2019, Cau *et al*., 2020, Hara *et al*., 2020, Martinelli *et al*., 2021). Similarly, no beads were recorded in a study on another commercially important crustacean, the brown shrimp *Crangon crangon,* from coastal waters off the Southern North Sea and Channel area (Devriese *et al*., 2015). Most microbeads have been recorded floating in surface waters rather than being transported to benthic sediments (Corcoran *et al*., 2020, Frias *et al*., 2020).

Results of this novel study show that MP fibre retention time in *N. norvegicus* is size dependent. Smaller MP fibres (3 mm) were excreted by t24, however, longer MP fibres were retained for longer, with fibres of 5 mm and 10 mm in length still being detected in *N. norvegicus* stomachs by t72 and t96, respectively. The retention of longer MP fibres may be attributed to the complex digestive tract of *N. norvegicus*, containing a foregut with chitinous plates that narrow towards the entrance to the hindgut (Murray and Cowie, 2011, Welden and Cowie, 2016a), which may slow down the egestion rate of the larger MP fibres. *N. norvegicus* have the capability to ingest solid particles of up to 20 mm in length and 4 mm in width as reported by Yonge (1924). Based on the results of the current study, the authors hypothesise that fibres larger than 5 mm may be too large to immediately pass-through *N. norvegicus* GIT. This hypothesis is in alignment with studies on MP ingestion for different species, such as the Atlantic cod, *Gadus morhua*, where 5 mm plastic beads were retained in the stomach for a longer period than the 2 mm beads (dos Santos and Jobling, 1991), and with the Sea scallop, *Placopecten magellanicus,* which retained 20 µm beads for a longer period than the smaller 5 µm beads (Brillant and MacDonald, 2000).

Nevertheless, other studies show contradictory findings, likely due to differences between species, MP sizes, concentrations, polymer type and shape, and therefore, do not allow for a direct comparison (Yu *et al*., 2021). Previous long term exposure trials for the same species, have focussed on detrimental effects of MPs rather than retention rate determination (Welden and Cowie, 2016b) . In contrast to our findings, many studies illustrate that smaller plastic particles, particularly nanoplastics are retained for longer periods of time (Lu *et al*., 2016, Crooks *et al*., 2019, Zeytin *et al*., 2020) which may reflect the ability of such smaller particles to translocate into different tissues and organs (Rezania *et al*., 2018, Weis and Palmquist, 2021). The use of microbeads in laboratory experiments also does not allow for a direct comparison with the natural environment as fibres are more commonly found in natural environments than beads (Rezania *et al*., 2018, Weis and Palmquist, 2021). Many studies have also carried out acute experiments with high MP concentrations, sometimes several orders of magnitude above environmentally relevant concentrations (Bour *et al*., 2020); therefore, caution must be taken into consideration while interpreting the results of such studies (Rebelein *et al*., 2021). Environmentally relevant concentrations of MP fibres were selected for this short-term experiment, based on previous results from Hara *et al*., (2020); Joyce *et al*., (2022) for the North East Atlantic (i.e., ~ 2 MPs individual^{-1}).

Previous studies have hypothesised that the removal of these MPs may be a result of either fragmentation of particles during digestion (Cau *et al*., 2020) or by ecdysis (Welden and Cowie, 2016a) and are therefore unlikely to accumulate in the GIT. It has been proposed that *N. norvegicus* and shore crabs (*Carcinus maenas*) have the ability to fragment and therefore reduce the size of MP fibres during digestion as a result of the grinding process of their gastric mill (Watts *et al*., 2015, Cau *et al*., 2020). However, this study could not support these claims as all MPs were found in the stomach with no MPs identified in the intestine. It has also been suggested that MP aggregations are excreted through ecdysis (moulting process) with previous studies showing lower levels of MPs recorded in the stomachs of individuals that had recently moulted, and fibres identified in the discarded gut lining of moulted individuals (Welden and Cowie, 2016a). Interestingly in a study conducted by Yu *et al*., (2021) looking at MP retention in barnacle naupliar larvae, organisms from muddy shores had a shorter retention time of MPs than those on rocky shores and coral reefs. This study suggests that the organisms from these muddy habitats normally egest non-food items, such as clay and stones at a faster rate, and due to this tolerance may similarly recognise and egest MPs as a non-food item (Yu *et al*., 2021).

The occurrence of entangled balls of fibres reported here is in alignment with other studies which have reporting MP entanglements in the stomachs of *N. norvegicus* (Murray and Cowie, 2011, Welden and Cowie, 2016a, Hara *et al*., 2020, Carreras-Colom *et al*., 2022). It has been suggested that the gastric mill of *N. norvegicus* is not designed to cut flexible resilient materials such as plastics, leading to the formation of these entangled balls due to the churning action within the stomach (Murray and Cowie, 2011). Previous studies have focused on the presence of these aggregations within the GIT of *N. norvegicus* (Welden and Cowie, 2016b, Carreras-Colom *et al*., 2022); however, there is no clear indication of the time taken to form these entanglements or how long they can be retained. In this study, introduced fibres were retained in existing entanglements as early as 24 hours after ingestion for 10 mm fibres and 72 hours after ingestion for 5 mm fibres. This highlights the potential for larger fibres to get caught up in entanglements and be retained for a longer period, however, such occurrences were only observed three times throughout the trials. Similarly, a recent study on *N. norvegicus* by Carreras-Colom *et al*., (2022) found that individuals with entanglements present in their stomachs had a larger quantity of longer fibres than those with no entanglements present.

The limitations of this study due to presence of unintroduced MPs in the GIT of *N. norvegicus* is acknowledged. These MPs may have originated from manmade seawater and has previously been recorded in salt (Peixoto *et al*., 2019), and/or may have entered the GIT prior to the depuration period. and may have played a role in the overall egestion rate due to entanglement or blockages in the stomach, however they were not the main focus of this retention study, and therefore were not examined. Nonetheless, unintroduced fibres were present in all trials, with the retention of introduced fibres only observed in trials with longer fibres (5 mm and 10 mm) indicating that the unintroduced MPs did not interfere with retention in the 3 mm trial. Where introduced fibres were retained in the entanglement (5 mm, *n*=1; 10 mm, *n*=2) this may result in longer retention times in comparison to the individuals without entanglements, had the trial exceeded 72 and 96 hours respectively. Furthermore, the number of individuals representing different sexes, sizes and moult stages was limited due to organisms available at sampling, and a larger sample size with wider variety of sexes and ages is thus recommended to accurately determine retention times for this species.

3.6 Conclusion

This study demonstrates the ability of *N. norvegicus* to actively ingest MP fibres of different lengths, however, no bioaccumulation was recorded. *N. norvegicus* can rapidly egest smaller MP fibres of 3 mm when exposed to environmentally relevant levels of MP contamination, potentially showing no negative effects during short term exposure. Larger plastic fibres (5 mm) and (10 mm) were not egested at the same rate as smaller MP fibres (3 mm), therefore suggesting that the retention time of MP fibres is size-dependent. *N. norvegicus* in the wild are exposed to many varied shapes, sizes, types, and polymers. Thus, further research is required to determine the retention times of other polymers, of different shapes and sizes, and of smaller plastics particles, e.g., nanoplastics, as these could potentially translocate within the organism and be retained for longer periods of time.

CRediT authorship contribution statement

Haleigh Joyce: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft. Róisín Nash: Conceptualization, Methodology, Writing – review & editing, Supervision. João Frias: Conceptualization, Methodology, Writing – review & editing, Supervision. Fiona Kavanagh: Writing – review & editing, Supervision. Jonathan White: Writing – review & editing. Thomas Power: Investigation.

Declaration of competing interest

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

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4. A proposed pan-European monitoring scheme for *Nephrops norvegicus* as a bioindicator for microplastic pollution

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Research highlights:

- Abundances of microplastics in *Nephrops norvegicus* vary across Europe.
- *Nephrops norvegicus* is a potential bioindicator for microplastic pollution.
- A traffic light system is proposed to reflect microplastic abundances on a Pan-European scale.
- A detailed monitoring programme using *Nephrops norvegicus* is presented.
- Sediment is proposed as a complementary matrix for microplastic pollution.

4.1 Abstract

Microplastics are a major global concern due to their ubiquity and heterogeneity in the marine environment. The use of marine biota as bioindicators has been highlighted by many researchers as a method of providing imperative data on MP pollution in the sea. At present there has been no marine organism assigned as a bioindicator for microplastics. This research reviews the current data available on the presence of microplastics in *Nephrops norvegicus*, a commercially important seafood species, highlighting the advantages and limitations of the species to determine its potential use as a bioindicator species for microplastic pollution. *N. norvegicus* fulfils the main ecological and biological selection criteria for microplastic bioindicator species. The decapod crustacean is a mainly sessile organism reflecting local levels of microplastic pollution and could/can define spatial and temporal variations in MP contamination, a prerequisite of a bioindicator. At present, there is no harmonized and standardised methodologies for microplastic analysis available, therefore, this research has proposed a detailed microplastic monitoring programme integrating the use of both *Nephrops norvegicus* and sediment as a monitoring tool on a European scale. The proposed monitoring programme outlines the protocol for sampling, methodology, reporting and the proposal of a new traffic light system to reflect the abundances of MPs and could provide an indication of areas at risk

of high MP pollution. Given the complexity of microplastics present in the marine environment, the authors recommend a more holistic approach with the integration of *Nephrops norvegicus* and sediments along with other species and matrices to cover all ecosystem compartments to provide a comprehensive database of MP levels and trends in the marine environment.

4.2 Introduction

Several governmental agencies, non-governmental organisations, and academic institutions worldwide have a vested interest in monitoring ecosystem health (Burger 2006, EC 2008), which in recent years, has led to the increased need for the development and proposal of bioindicator species (Fossi *et al*. 2018, Cau *et al*. 2019, Macali and Bergami 2020). Bioindicator species are often used to assess the quality of environmental health as well as to forecast changes resulting from anthropogenic pressures (Holt and Miller 2011). Monitoring, based on bioindicator species, not only provides information on the state of the environment, but it also has the potential to assess environmental change (Lawton and Gaston 2001, Parmar *et al*. 2016) while serving as a barometer for environmental pressures (Smeets and Weterings 1999, Maxim *et al*. 2009). The use of an appropriate single species as an ecological bioindicator for monitoring contamination has many benefits including reliability and cost-effectiveness (Parmar *et al*. 2016, Siddig *et al*. 2016). The rationale for selecting a single bioindicator species, however, is often lacking, with little justification provided (Siddig *et al*. 2016, Wesch *et al*. 2016). To be successful, a bioindicator species should be common and widely distributed; be well studied in terms of its biology and ecology; be of ecologic relevance and/or commercially importance; be easy to access and inexpensive to survey; and most importantly be reflective of its surroundings, particularly when in the presence of xenobiotics, contaminants, and pollutants (Holt and Miller 2011, Kershaw *et al*. 2019).

Many bioindicator species are used worldwide to monitor changes in their surrounding environment, for example, phytoplankton are a common bioindicator species used for water quality analysis on a global scale (Devlin *et al*. 2012, Jiang *et al*. 2014, Pourafrasyabi and Ramezanpour 2014). Bivalve molluscs are another extensively used bioindicator, with mussels (*Mytilus spp*.) being the most commonly used in numerous monitoring programmes. For example, the Mussel Watch Programme, is the longest-running contaminant monitoring program in U.S waters, monitoring inorganic and organic contaminants (Viñas *et al*. 2012), and is even in use in Europe (Thébault *et al*. 2008). The OSPAR Coordinated Environment Monitoring Programme (CEMP) measures the concentrations of trace metals such as mercury (Hg), cadmium (Cd), lead (Pb); polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and other pollutants in biota using mussels (OSPAR 2022b). Other fish and shellfish species are also used under OSPAR CEMP guidelines including Dab (*Limanda Limanda*) for the monitoring of PCBs in biota (OSPAR 2022c).

Plastic pollution has been recognised as a contaminant of emerging concern in the marine environment, with recent monitoring efforts put in place to reduce marine litter (Wenneker and Oosterbaan 2010, van Franeker *et al*. 2021, OSPAR 2022a). The monitoring of beach litter and seafloor litter is currently being carried out in the OSPAR Maritime Area under descriptor 10 of the MSFD which aims to protect the marine environment in relation to marine litter "*Properties and quantities of marine litter do not cause harm to the coastal and marine environment*" (EC 2008). An example of a bioindicator species for the monitoring of plastic levels and trends in the North Sea is the Northern Fulmar (*Fulmarus glacialis*), which is used to quantify the spatial and temporal patterns and trends of floating plastic abundances (Van Franeker *et al*. 2011). The monitoring of plastics retrieved from the stomach of beached seabirds is carried out under the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) system of Ecological Quality Objectives (EcoQOs) which has been included in the MSFD (van Franeker *et al*. 2021). The stomach contents of the loggerhead turtle (*Caretta caretta*) have also been proposed to assess plastic pollution to reach Good Environmental Status (GES) by the EU MSFD (Domènech *et al*. 2019).

There have been many species proposed as potential bioindicators, however, none have yet been assigned as a bioindicator for microplastic (MP) contamination at a European level. Proposed bioindicator taxa for MP contamination across the globe include fish (Bray *et al*. 2019, Garcia-Garin *et al*. 2019, Kılıç and Yücel 2022), crustaceans (Xu *et al*. 2020, Carreras-Colom *et al*. 2022), and molluscs (Beyer *et al*. 2017, Ding *et al*. 2021); however, regular monitoring of MP contamination is still missing on both a national and European level. Jellyfish have been suggested as bioindicators for MP pollution on a global scale (Macali and Bergami 2020) along with mussels (Beyer *et al*. 2017, Li *et al*. 2019). Mussels are the most widely recommended (Beyer *et al*. 2017, Li *et al*. 2019) owing to their status as a bioindicator for a variety of contaminants because of their global distribution, ease of sampling, feeding strategy, high tolerance to a wide variety of environmental parameters and potential link to MPs entering the human food chain (Van Cauwenberghe and Janssen 2014, Li *et al*. 2016, Li *et al*. 2019). Many countries are considering MP monitoring on specific species such as mussels (Bråte *et al*. 2017, Kershaw *et al*. 2019) however, these organisms would likely be restricted to coastal areas (Beyer *et al*. 2017, Li *et al*. 2019). Nevertheless, it must be noted that no single species can cover all environmental matrices, therefore it is appropriate to consider a holistic monitoring approach and target programmes monitoring other compartments such as sediment and benthic organisms as additional management tools (Lusher *et al*. 2017, Bonanno and Orlando-Bonaca 2018, Pagter *et al*. 2020).

This research will focus on the suitability, feasibility and limitations associated with *N. norvegicus* as a bioindicator species for MP contamination within Europe. Currently there is no organism or scientifically agreed threshold values relating to MP ingestion established (DHLGH 2021). The aim of the study is to address this knowledge gap and to (i) determine the ability of *N. norvegicus* to be used as a bioindicator species (ii) assess the effectiveness of *N. norvegicus* as a bioindicator species for microplastic pollution and (iii) propose a methodological approach for the assessment of MPs using *N. norvegicus* as a bioindicator species for MP pollution. The results will help to inform policy makers and provide a structure for the implementation of a MP monitoring program in respect of the Marine Strategy Framework Directive (EC 2008) or any future European or national regulations on MPs.

4.3 European field investigations on MP contamination in *Nephrops norvegicus*

All data on *N. norvegicus* (Figure 1) used in this study was collected as part of baseline MP loadings in *N. norvegicus* and the surrounding sediment by Joyce *et al*. (2022) along with desk work of previous research carried out in Europe to determine the abundance of MPs. A review of the documented MP ingestion by *Nephrops norvegicus* was collected as part of this study to determine the abundance of MPs in *N. norvegicus* across Europe. A thorough literature review was conducted by searching various academic databases including Web of Science and Google Scholar published between 2011 and 2022. The following terms were using in searching databases: (microplastics or microplastic and *Nephrops* or *Nephrops norvegicus* or Norway lobster or Dublin Bay prawn). Lists of references from the research papers were inspected in the aim of finding studies that had not been retrieved from the search platforms. This review included 11 articles that reflected MP abundances within wild caught *N. norevegicus* across Europe (Table 1).

Fig. 1. Male *Nephrops norvegicus* (Credits: Haleigh Joyce, 2022)

Table 1. Literature review Summary on microplastic analysis in *N. norvegicus*. (Methods of examination: A: Alkaline digestion (10% KOH) + µFTIR; B: Visual examination + µFTIR; C: Visual examination + µRAMAN; D: Visual examination; E: Enzymatic digestion + µFTIR; F: Density Separation (NaCl) + µFTIR; G: Density separation + Alkaline digestion + µFTIR; H: Density Separation, + Hydrogen peroxide digestion (H2O2) + µFTIR; n.l.: not listed; n.d.: no data).

Location	Year	N	Method of examination	Abundance items ind^{-1}	% Occurrence	Most common MP type	MP size range (mm)	Most common length (mm)	Max. MPs ind^{-1}	Entanglements reported	Reference
North East Atlantic	2020	600	A	2.20 ± 2.47	72	Fibre	0.045 - 53.88	<1	19	Yes	Joyce et al., 2022
Western Irish Sea	2020	100	A	3.66	82	Fibre	n.l.	n.l.	16	nl	
Porcupine Bank	2020	100	A	0.8	42	Fibre	n.l.	n.l.	7	nl	
Aran Prawn Grounds	2020	100	A	2.31	84	Fibre	n.l.	n.l.	13	nl	
SE and SW Coasts of Ireland	2020	100	A	2.15	77	Fibre	n.l.	n.l.	8	nl	
Labadie Jones and Cockburn	2020	100	A	1.56	64	Fibre	n.l.	n.l.	19	nl	
The Smalls	2020	100	A	2.74	81	Fibre	n.l.	n.l.	11	nl	
Clyde Sea, Gulf of Cadiz and Baleric Sea	2007-2019	204	B	7.60 ± 12.01	77.8	Fibre	0.1 - 44.7	1 -2	75	Yes	Carreras-Colom et al., 2022
Clyde Sea	2019	60	B	7.00 ± 11.90	n.l.	Fibre	n.l.	n.l.	n.l.	Yes	
Gulf of Cadiz	2017	24	B	13.08 ± 13.49	n.l.	Fibre	n.l.	n.l.	n.l.	Yes	
Costa Brava	2019	20	B	6.20 ± 6.80	n.l.	Fibre	n.l.	n.l.	n.l.	Yes	
	2018	20	B	2.50 ± 2.50	n.l.	Fibre	n.l.	n.l.	n.l.	Yes	
Barcelona	2019	20	B	12.55 ± 20.78	n.l.	Fibre	n.l.	n.l.	n.l.	Yes	
	2018	20	B	10.40 ± 14.08	n.l.	Fibre	n.l.	n.l.	n.l.	Yes	
	2007	20	B	9.40 ± 13.36	n.l.	Fibre	n.l.	n.l.	n.l.	Yes	
Ebro Delta	2019	20	B	5.20 ± 4.44	n.l.	Fibre	n.l.	n.l.	n.l.	No	
	2018	20	B	2.15 ± 2.76	n.l.	Fibre	n.l.	n.l.	n.l.	No	
Adriatic Sea	2019	23	E	4.9 ± 2.4	100	Fragments	0.051 - 0.431	x̄ ~ 0.145	23	No	Martinelli et al., 2021
Sardinian waters, Mediterranean Sea	nl	27	F	2.1 ± 0.6 MPs and 3.9 ± 0.5 MPs in stomachs and intestines	100	Fragments	0.2 - 1	n.l.	13	No	Cau et al., 2020
Sardinian waters, Mediterranean Sea	2017	89	G	5.5 ± 0.8	83	Films	0.1- 5	< 0.5	42	No	Cau et al., 2019
Galway Bay West Coast of Ireland	2017	32	A	0.48	nl	Fibre	n.l.	n.l.	n.l.	No	Pagter et al., 2020
Irish Waters	2016	150	A	1.75 ± 2.01	69	Fibre	0.143 - 16.976	1 -2	10	Yes	Hara et al., 2020
Aran Prawn Grounds	2016	30	A	0.9 ± 1.03	56.7	Fibre	n.l.	n.l.	4	n.l.	
Bantry Bay	2016	30	A	1.67 ± 2.0	73.3	Fibre	n.l.	n.l.	10	n.l.	
Kenmare Bay	2016	30	A	2.3 ± 2.47	70	Fibre	n.l.	n.l.	10	Yes	
Magharees Union	2016	30	A	1.67 ± 1.9	60	Fibre	n.l.	n.l.	7	n.l.	
North Irish Sea	2016	30	A	2.20 ± 2.2	83.3	Fibre	n.l.	n.l.	9	n.l.	
Adriatic Sea	2016	10	H	1 ± 0	10	Fibre	n.l.	n.l.	n.l.	No	Avio et al., 2020
Balearic Islands	2015	8	D	0.63 ± 0.32	38	Fibre	n.l.	n.l.	n.l.	No	Alomar et al., 2020
Scottish waters	2011	1450	B	n.d.	67	Fibre	n.l.	n.l.	n.l.	Yes	Welden and Cowie 2016
Clyde Sea Area	2011	1000	B	n.d.	84.1	Fibre	n.l.	n.l.	n.l.	Yes	
North Minch	2011	150	B	n.d.	43	Fibre	n.l.	n.l.	n.l.	Yes	
North Sea	2011	300	B	n.d.	28.7	Fibre	n.l.	n.l.	n.l.	Yes	
Clyde Sea; Isles of Cumbrae	2009	120	C	n.d.	83	Fibre	n.l.	n.l.	n.l.	Yes	Murray and Cowie 2011

4.3.1 Geographical variation in MP abundance in Nephrops norvegicus across Europe

N. norvegicus, is widely distributed across Europe with stocks divided into Functional Units (FU) and Geographical Subareas (GSA) which are designated fishing grounds based off suitable muddy habitat for the species. The MP abundances in *N. norvegicus* stocks have been investigated in FU's 10, 11, 13, 15, 16, 17, 19, 20-21, 22 and 30 (Welden and Cowie 2016a, Hara *et al*. 2020, Joyce *et al*. 2022a) and in GSA's 5, 11, 17 (Cau *et al*. 2019, Martinelli *et al*. 2021, Carreras-Colom *et al*. 2022) across Europe (figure 2).

MP abundances and characteristics vary between field investigations, with various MP loadings recorded, potentially reflecting local levels of MPs in the surrounding environment. This variance may also occur due to diverse methodological techniques used; possibly due to cross-contamination when adequate quality control is not carried out; or due to sampling time/season. The occurrence of MPs in *N. norvegicus* varies spatially with 72 % (Joyce *et al*. 2022a) and 69% (Hara *et al*. 2020) of organisms recorded as carrying MPs (in their gastro-intestinal tract; GIT) in the North East Atlantic. Similarly, high MP prevalence levels were recorded in *N. norvegicus* in Scottish waters, namely 67% (Welden and Cowie 2016a) and 83% (Murray and Cowie 2011). In the Mediterranean Sea, 83% and 100% occurrence were recorded (Cau *et al*. 2019, Martinelli *et al*. 2021). Recent studies have identified areas within the sampling range that seem to be widely affected by MP contamination with higher abundances (e.g., Clyde Sea in Scottish waters, Gulf of Cadiz in the Northeast Atlantic, Western Irish Sea in Irish waters; see Table1); (Welden and Cowie 2016a, Carreras-Colom *et al*. 2022, Joyce *et al*. 2022a), which represents a potential and/or increased risk to marine biota. A clear difference can be seen between these regions/areas (Table 1), with the highest abundances reported in the Mediterranean and the Clyde Sea (Welden and Cowie 2016a, Cau *et al*. 2019, Martinelli *et al*. 2021, Carreras-Colom *et al*. 2022). Both these sites have been described as areas of high MP pollution (Cózar *et al*. 2015, Welden and Cowie 2016a). This provides further evidence of the suitability of using *N. norvegicus* as a bioindicator of MP contamination in the environment, displaying levels of ingested MPs that reflect the variation in MP availability between locations. As well as showing spatial trends, *N. norvegicus* have displayed their ability to detect temporal trends, where in a recent study, both annual and intra year variability was reported (Carreras-Colom *et al*. 2022).Organisms sampled in the Ebro Delta showed variations between 2018 and 2019 sampling events and specimens from the Clyde Sea, sampled in both May and August of the same year showed a great variation of levels between sampling dates (2.77 and 11.23 MPs per individual respectively) (Carreras-Colom *et al*. 2022). Moreover, wider temporal differences can be inferred through *N. norvegicus* when comparing the MP levels observed in the Clyde Sea from sampling events in 2009, 2011 and 2019 (Murray and Cowie 2011, Welden and Cowie 2016a, Carreras-Colom *et al*. 2022). However, the differences between the methodological approach from these studies does not allow for a direct comparison, therefore, caution must be taken into consideration while interpreting results.

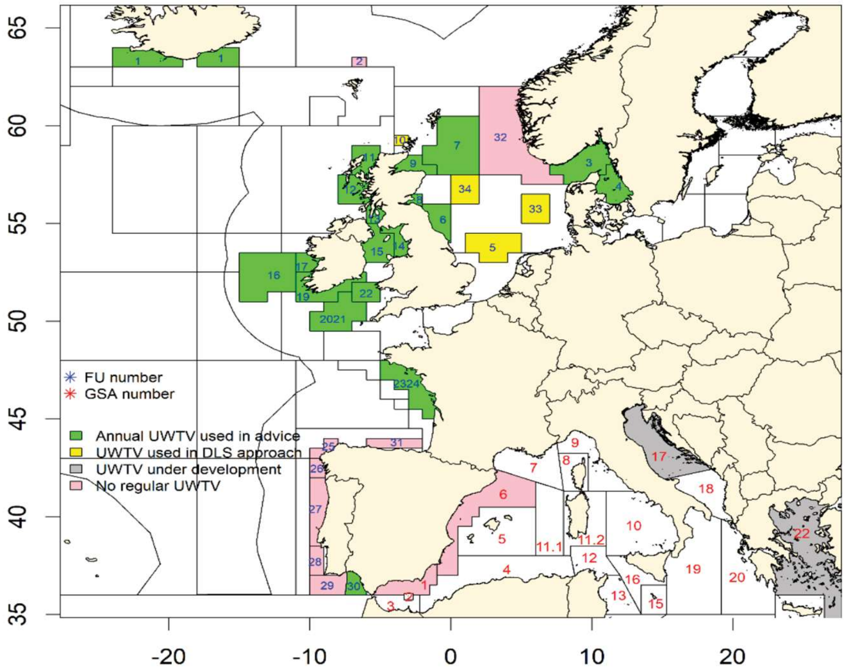

Fig. 2. *Nephrops norvegicus* distribution across functional units (FUs) in ICES areas and geographical subareas (GSAs) in the Mediterranean. Adapted from Dobby *et al*. (2021)

4.3.2 Characteristics of MP contamination in *Nephrops norvegicus*

Global patterns have identified fibres as being the most dominant MP type in the marine environment and the most observed MP type in *N. norvegicus* (Hara *et al*. 2020, Rebelein *et al*. 2021, Carreras-Colom *et al*. 2022) with fibres reported in eight out of the eleven reviewed literature. There are however contrasting studies, that targeted MP ingestion by *N. norvegicus* in the Mediterranean and have reported a predominance of fragments and films over fibres (Cau *et al*. 2019, Cau *et al*. 2020, Martinelli *et al*. 2021). The entanglements of fibres, which are thought to aggregate during the intermoult period, have been highlighted as a concern, due to their possibility of causing blockages and false satiation in organisms (Welden and Cowie 2016b), with five out of the 11 field investigations reporting the presence of entanglements (table 1). Furthermore, a recent study found a correlation between high levels of MP and the presence of entangled balls within *N. norvegicus* (Carreras-Colom *et al*. 2022). In another study, conducted by Welden and Cowie (2016a) organisms from the North Sea and the Minch had lower levels of MP contamination and were mainly comprised of single strands of fibres in comparison to the Clyde Sea area which had a higher abundance of MPs and entanglements. This supports the hypothesis that the low prominence of entanglements seen in the six FU's in the

North East Atlantic (Joyce *et al*. 2022a) reflect a low level of MP pollution in comparison to areas of high plastic pollution such as the Mediterranean. The most reported polymers included, polyethylene (PE), polypropylene (PP), polyamide (PA), polyester (PES)(PET), polystyrene (PS) and polyvinyl chloride (PVC) and polyacrylonitrile (PAN).

4.3.3 Laboratory exposure experiments of MPs in *N. norvegicus*

Laboratory exposure experiments play an important role in understanding the potential adverse effects of MP exposure to marine biota through ingestion (Murray and Cowie 2011, Watts *et al*. 2014, Welden and Cowie 2016b, Hankins *et al*. 2018, Rebelein *et al*. 2021). *N. norvegicus* has been previously used in laboratory exposure experiments to assess the uptake, retention, accumulation, and egestion of MPs (Murray and Cowie 2011, Welden and Cowie 2016b, Devriese *et al*. 2017, Joyce *et al*. 2022b). As non-selective scavengers no differentiation between food and food seeded with MPs (beads or fibres) has been recorded (Devriese *et al*. 2017, Joyce *et al*. 2022b).

Murray and Cowie (2011) was the first study to demonstrate the ingestion and retention of MP fibres in *N. norvegicus*. The short-term laboratory study used 20 organisms, 10 of which were fed five 5 mm PP fibres. After 24hrs all organisms were shown to contain MP fibres within their stomachs illustrating the possibility that MP fibres are not excreted at the same rate as natural food items which have been shown to be egested within a 24 h time frame (Sardà and Valladares 1990, Joyce *et al*. 2022b). Another short-term exposure trial focused on the effects of both virgin and PCB loaded microbeads (6-600 µm) over 3 weeks; the beads were seen to have no impact on the nutritional state of *N. norvegicus.* In contrast, a long-term exposure trial carried out on 36 individuals, in which 12 organisms were fed 3-5 mm PP fibres over an 8-month period revealed a decrease in the nutritional state and false satiation (Welden and Cowie 2016b). This highlights the potential of fibres at high abundances to become entrapped in the GIT in comparison to beads which are spherical and therefore may be easily egested (Yu *et al*. 2021).

A recent exposure trial carried out by Joyce *et al*. (2022b) determined the size dependent egestion on polyester MP fibres of different lengths and found that smaller fibres of 3 mm were egested within 24 hrs. However, larger fibres of 5 and 10 mm were retained for longer. This is similar to findings by Carreras- Colom *et al*. (Carreras-Colom *et al*. 2022) where longer fibres were more dominant in entanglements found in the stomach of *N. norvegicus*. The research suggests that although larger fibres seem to be retained for a longer period and can potentially become entangled, they are not believed to accumulate over their entire life as in previous studies where the larger (presumed to be older) organisms did not have a higher abundance of MPs in comparison to smaller individuals (Murray and Cowie 2011, Welden and Cowie 2016a, Carreras-Colom *et al*. 2022, Joyce *et al*. 2022a). Therefore, it has been hypothesised that MP particles are either eventually egested, fragmented, or removed through ecdysis (Welden and Cowie 2016a, Cau *et al*. 2020, Joyce *et al*. 2022a). These results suggest that *N. norvegicus* are effective at monitoring smaller MP particles <3 mm which are thought to be recent MP contamination and are the most reported sizes in *N. norvegicus* (Cau *et al*. 2019, Hara *et al*. 2020, Carreras-Colom *et al*. 2022, Joyce *et al*. 2022a). However, they may potentially retain and/or entangle fibres of greater lengths present at high abundances, potentially giving a false representation of the MP currently available in the environment with more research needed to understand the retention of MP fibres.

The behaviour of smaller MP particles and nanoplastics in *N. norvegicus* is still largely unknown except for one recent study which identified MPs in the edible tissue (Martinelli *et al*. 2021), highlighting the possibility of smaller MPs and/or nanoplastics to translocate.

4.4 *Nephrops norvegicus* and its potential use as a bioindicator species in Europe

The ongoing monitoring practices for MP pollution are limited especially in term of their distribution and temporal trends. The lack of data especially in open oceans and remote areas are required to form baselines and understand the long-term trends and effects of MP pollution. The assessment of MPs in the marine environment has been increasing rapidly with particular interest given to the commercially important seafood species *N. norvegicus* in recent years (Cau *et al*. 2019, Hara *et al*. 2020, Martinelli *et al*. 2021, Carreras-Colom *et al*. 2022, Joyce *et al*. 2022a).

4.4.1 Advantages of using *Nephrops norvegicus* as a bioindicator species

According to Table 2, the characteristics support the use of *N. norvegicus* as a bioindicator species for the monitoring of MP contamination. *N. norvegicus* are relatively sessile organisms, making them a suitable bioindicator species, as they can reflect local levels of MP contamination in other matrices (e.g., sediment) (Kershaw *et al*. 2019, Carreras-Colom *et al*. 2022), providing clear spatial and temporal gradients. They are a commercially valuable seafood species with landings worth approximately €287 million in European waters in 2018 (Eumofa 2020). In areas of high seafood consumption in Europe, 11,000 MP particles are estimated to be ingested by humans annually (Van Cauwenberghe and Janssen 2014), therefore, they play a valuable role in assessing the potential link to human health effects. Moreover, no effects of the ingestion of MPs have been assessed to date in terms of human health impact. Organisms that are consumed whole with their GIT intact pose a greater risk of MP transfer to humans in comparison to *N. norvegicus* as the digestive tract is usually removed prior to consumption. The non-edible GIT in decapod crustaceans is suggested to be the main area of MP retention (Yin *et al*. 2022). Nonetheless, these organisms can still be used for monitoring purposes from a human health perspective (Kershaw *et al*. 2019).

Table 2. The suitability of *N. norvegicus* as a bioindicator for microplastic contamination was assessed using the six main ecological and biological selection criteria set out by Fossi *et al*. (2018).

Background information	• Common name: Norway lobster, Scampi, Dublin Bay prawn, Langoustine (Figure 1.) • Classification: Decapod crustaceans from the family Nephropidae, sub-family Nephropinae • Maximum total length of 25 cm • Life expectancy of 5 – 10 years	Hill 2008, Ungfors *et al*. 2013
Habitat type/vagility	• Benthic organism • Found in muddy environments, on suitable sediment for burrowing • Found at depths ranging between 10 - 800 m • Rather sessile, territorial organisms, not moving far from their burrows, only leaving to forage and mate.	Rice and Chapman 1971 Hill 2008 Ungfors *et al*. 2013 Welden and Cowie 2016b Lolas and Vafidis 2021
Trophic information and feeding behaviour	• Scavenger species • Opportunistic predation and suspension-feeding also observed • Non-selective feeding behaviour and have been recorded to ingest non-food materials • Mid Trophic level	Santana *et al*. 2020, Parslow-Williams *et al*. 2002, Murray and Cowie 2011, Walkinshaw *et al*. 2020
Spatial distribution	• Wide distribution, ranging throughout the eastern Atlantic region, from Iceland to Norway to the Atlantic coast of Morocco and in the Mediterranean (Figure 2)	Ungfors *et al*. 2013, Martinelli *et al*. 2021, Joyce *et al*. 2022a
Commercial importance and conservation status	• Landings worth approximately €37 million in 2020 in Ireland • In 2010, the total landings (European fisheries) were 66,500 tonnes. • The consumption of *N. norvegicus* is recorded in many geographical locations	Ungfors *et al*. 2013, Marine Institute 2021
Documented ingestion of marine litter	• MPs have been recorded in *N. norvegicus* from different geographical locations • Retention experiments of microplastics carried out • Adverse effects reported	Murray and Cowie 2011, Welden and Cowie 2016b, Cau *et al*. 2019, Cau *et al*. 2020, Hara *et al*. 2020, Martinelli *et al*. 2021, Carreras-Colom *et al*. 2022
Others	• Easy to collect due to ongoing monitoring and commercial exploitation of stocks – opportunistic sampling • Human consumption - valuable role in MP and associated contaminants transfer from seafood into the food chain • Similar types, colours, sizes and polymers retrieved from both *N. norvegicus* and surrounding sediment	Welden 2015, Joyce *et al*. 2022

N. norvegicus are opportunistic scavengers positioned at mid trophic level and found at various depths (10-800 m) in muddy benthic environments. This non-selective feeding behaviour and its position in the food chain are potential reasons for MP ingestion by this species (Murray and Cowie 2011, Walkinshaw *et al*. 2020). A recent study investigating the abundance of MPs in two crustaceans, *N. norvegicus* and *Aristeus antennatus,* reported that *N. norvegicus* had a higher abundance of MPs which was likely due to their feeding behaviour (Cau *et al*. 2019). Their wide geographical and depth distribution allow for comparison between areas across Europe (Carreras-Colom *et al*. 2022). These decapods are easily accessible for routine sampling or fishing and are practical for MP analysis in a laboratory setting (Kershaw *et al*. 2019, Novillo *et al*. 2020).

4.4.2 Limitations of using Nephrops norvegicus as a bioindicator species

Like all management tools, there are limitations associated with the use of *N. norvegicus* as a bioindicator species. There are still knowledge gaps to be addressed such as a more complete understanding of the retention times of MPs of different sizes, shapes, and polymers. The retention time of different sized MPs was investigated by Joyce *et al*. (2022b) in *N. norvegicus* proposing that retention may be a snapshot of what was recently consumed in terms of smaller MP particles (3 mm) or may be retained over a long period of time for larger MPs 5-10 mm. It was assumed that larger fibres may become entrapped and at high abundances may become entangled in the GIT of *N. norvegicus* and therefore this retention time could potentially be as long as 6 months (common moult period for males) and 12 months (moult for females) or eventually fragmented and/or egested (time frame unknown) (Welden and Cowie 2016a, Cau *et al*. 2020). The retention time and harm caused to organisms is not determined during monitoring programmes/field investigations, therefore laboratory studies will need to be conducted to better understand the potential effects of MPs and strengthen the role of *N. norvegicus* as a bioindicator species. Nevertheless, the benefits of this species as a monitoring tool outweighs the limitations, lending themselves well as an indicator species of environmental contamination owing to their availability, spatial and depth distribution, interactions with seafloor sediment and position in the ecosystem and food chain.

4.5 Proposed monitoring programme

A pan-European approach to monitoring is recommended where representative samples from the key *N. norvegicus* FUs and/or GSAs are collected to reflect the geographical spread of *N. norvegicus*. The proposed protocol for monitoring MP contamination will take a twofold approach (Figure 3) as suggested by Joyce *et al*. (2022), relating to the MP loadings in *N. norvegicus* and the presence of MPs in the surrounding seafloor sediment informing both D10C2 (amount of micro-litter in the marine and coastal environment) and D10C3 (impacts of micro-litter on marine species) under the MSFD (2008/56/EC) (EC 2008).

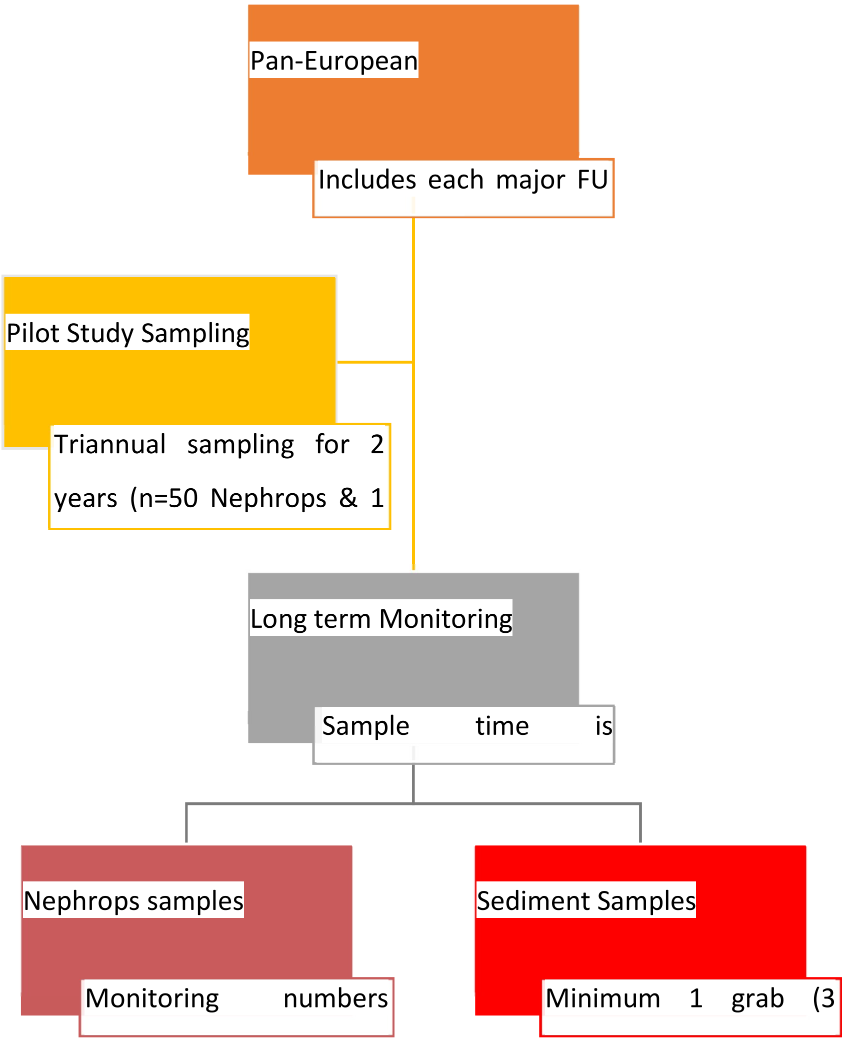

Figure 3. Outline of the proposed sampling for a Pan-European MP monitoring programme. Monitoring to be carried out on each major FU/GSA. Pilot study sampling to take place three times per year over the first two years. Long term monitoring of *N. norvegicus* and sediment taking place thereafter depending on the conclusions of the pilot study.

N. norvegicus and sediment sampling would be carried out in conjunction with regular national sampling programmes that are currently in existence in the North East Atlantic and across the rest of Europe, from which *N. norvegicus* samples and benthic sediment samples can be easily obtained, e.g., the Irish Marine Institute fisheries Sampling and Data Collection programme, the Irish Marine Institute *Nephrops* Under Water Television Surveys and the MEDiterranean International Trawl Survey (MEDITS). After measurements are taken for stock assessment purposes, organisms can be repurposed for MP analysis. In the case where organisms through existing sampling programmes are not available, organisms should be obtained from commercial fisheries which are largely year-round with the exception of fishery closures e.g., in the North East Atlantic the Porcupine Bank (FU16) is closed during May.

Monitoring will be carried out three times a year for the first two years to establish baselines and to get intra-year variability, with annual or biannual sampling taking place after, depending on the trends and variations in MP abundances established.

4.5.1 Samples and sampling

Nephrops norvegicus in combination with the sedimentary habitat are proposed for the initial monitoring of MPs. Sampling sites need to be considered on both a pan-European and a national level with locations selected to represent the variability within a water body and/or consisting of a range of FUs/GSAs e.g., four of the primary FUs could represent Irish waters, namely, the Western Irish Sea (FU15), the Porcupine Bank (FU16), Aran Prawn Grounds (FU17) and one FU from the Celtic Sea Area e.g., Labadie Jones and Cockburn (FU20-21).

Nephrops

To represent each sampling site covering a water body/FU/GSA, the pilot study sample size of at least 50 organisms will be collected three times a year, following the ongoing sampling timetable, to assess the level of MPs present following the guidelines set out by the MSFD (Directive 2013, Hermsen *et al*. 2018) for the first two years. The long-term monitoring programme will be carried out with a possible reduced sample size based on the power analysis of the pilot study and where no intra-year variability occurs the samples will be collected annually for long term monitoring or biannually if no annual variations are low.

Size, sex, and moult stage of organisms should be representative of those of a commercial catch and randomly selected. There is no indication that a balance in sexes is required for monitoring based on current research. Once caught, organisms should be frozen -20 °C (Hermsen *et al*. 2018) until further processing to avoid egestion of further MPs.

Sediment

For sediment analysis, a minimum of one benthic grab sample will be taken per FU/GSA, from which three replicate subsamples will be taken. A minimum of three replicates is recommended to understand the variance and error around the data. Any benthic grab and corers normally used to collect marine sediment may be used. Three sediment subsamples from the top 5 cm within the grab will be collected using a stainless-steel core (e.g., 5 x 10 cm) to collect approx. 100 g sediment per replicate. Each sample should be placed into a separately labelled glass jar. If samples are not being processed immediately, they should be frozen at -20 °C. Sediment samples from the FU/GSA should be taken in the same location and time frame as *N. norvegicus* samples where possible.

4.5.2 Methodology and Meta data

Metadata should be collected in a structured and formal manner to allow reliable assessments to be made. Information to be collected includes:

- Location data, date, and time of sampling (GPS coordinates, depth, environmental conditions),
- Equipment used and collection method (e.g., corers, nets, creel),
- Name and type of vessel
- Number of specimens collected from each site

Detailed MP analysis of *N. norvegicus* and benthic sediment are carried out using best practice protocols as described by Joyce *et al*. (2022) (Table 3.). Organisms would be defrosted, and the exterior rinsed with ultra-pure water. Prior to dissection, each specimen's sex, carapace length (CL), weight (excluding chelipeds), physical damage, carapace hardness and moult stage are to be recorded (additional information can be included such as total length, width, and reproductive state). Sex of each organism can be determined by the structure of the sexual pleopods (Farmer 1974). Carapace hardness to be defined following Milligan *et al*. (2009) divided into categories of hard, soft and jelly. Moult stage is then classified based on the carapace hardness category (Milligan *et al*. 2009, Murray and Cowie 2011). Ridgway *et al*. (2006) proposed a three category damage index that categories the structural damage to *N. norvegicus* as (a) no damage, (b) lightly and (c) heavily damaged.

Extraction: Detection and quantification protocols of MPs in *N. norvegicus* will follow the methodology recommended by Hara *et al*. (2020) and applied by Joyce *et al*. (2022a). Digestion of the GIT including the foregut, midgut and hindgut is carried out using a 10% potassium hydroxide (KOH) in an oven at a maximum temperature of 40 °C for 48 hours (Bessa *et al*. 2019, Hara *et al*. 2020). Extraction of MPs from sediment is carried out using density separation methods, using high density solutions such as Sodium Tungstate Dihydrate ($Na_2WO_4 \cdot 2H_2O$) solution (41% w/v; 1.4 g/cm^3) as recommended by Pagter *et al*. (2018).

Particle analysis: Visual examination of MPs are carried out under a stereoscopic microscope where MPs are counted, measured, and photographed. The size (length, width, and area), colour and type of MPs are recorded. Visual sorting of MPs alone is not recommended because small particles are visually difficult to detect in organic rich samples and the possibility of natural fibres present in samples (Wesch *et al*. 2016, Pagter *et al*. 2020). Chemical characterisation of MPs is to be carried out using spectroscopic methods for example Fourier Transform Infrared Spectroscopy (FTIR), Laser Direct Infrared (LDIR) or Raman Spectroscopy.

Table 3. Proposed methodology for MP analysis in *N. norvegicus* and benthic sediment samples (for further explanation see referenced methods).

	Collection Method	Collected samples	MP Extraction process	Processing
Nephrops norvegicus	For example: Pots Creels Beam Trawl Bottom Trawl	Samples frozen (-20 °C)	Digestion using 10% potassium hydroxide (KOH; CAS 1310-58-3)	Filtration, visual sorting, and chemical characterisation
Sediment	For example: Day Grab Van Veen Grab Box corer Corer Shipek grab	5mm depth (Samples frozen ; -20 °C; optional)	Density separation using Sodium Tungstate Dihydrate ($Na_2WO_4{\cdot}2H_2O$; CAS 10213-10-2) solution (41% w/v; 1.4 g/cm^3)	Filtration, visual sorting, and chemical characterisation

Laboratory Contamination control - Cross–contamination will be reduced by using a 100% cotton lab coat and nitrile gloves. Wearing of synthetic or semi - synthetic clothing under the lab coat should be avoided where possible. Decontamination of glassware should be carried out using dilute (10%) Nitric Acid (HNO_3; CAS 7697-37-2), followed by rinsing three times using ultra-pure water and left to dry upside down to avoid accumulation of airborne particles. All surfaces are cleaned prior to use. Air controls should be used every day during all stages of processing using microfibres filters (Whatman GF/C) to monitor airborne MP particles. Procedural blanks are carried out on ultra-pure water, and all solutions, as well as airborne particles, to monitor and account for potential contamination.

4.5.3 Reporting

Reports should include the following for each waterbody and/or FU:

- The total number of organisms and sex
- Biometric measurements (weight, carapace length, total length)
- Tissues processed for the analysis (e.g., GIT)
- The abundance of MPs retrieved (synthetic, semi-synthetic, and natural)
- The % occurrence of organisms containing MPs
- MP characteristics including size, shape, colour and polymer characterisation
- The mean abundance and size ranges (with confidence intervals and quartiles) of MPs per individual by FUs/GSAs
- The highest MP abundance recorded in one individual
- The presence and % occurrence of micro fibre entanglements
- The abundance of MPs (with confidence intervals and quartiles) from procedural blanks and air controls,

- MP abundance in sediment by FUs/GSAs, reported as MP/kg (dry weight) with confidence intervals.
- Status of the water body and/or FU/GSA in relation to MPs (applying the Traffic light system proposed in Fig 5).

4.5.4 Status of the Water Body

Nephrops

The use of *N. norvegicus* as a monitoring tool is to be used on the basis of a new traffic light system as proposed by Joyce *et al*. (2022a) (figure 4).

- **Green**: The system reports levels of low/green MP contamination if the abundance is < 2 MPs per individual for 75% of the sample.
- **Orange**: The levels of MP contamination are regarded as moderate/orange if the abundance is ≥ 2 but < 5 MP particles per individual for > 25%.
- **Red**: The levels of MP contamination are regarded as high/red if the abundance is ≥ 5 MP particles per individual for > 25%. Note: Where 20% of individuals record the presence of micro fibre entanglements (>5 MPs) then this area/site should be categorised as having high MP contamination (red).

This will reflect the abundance of MP and could provide an indication of areas at risk of high MP contamination. The long-term goal in terms of MP ingestion by *N. norvegicus* is having 75% of the individuals in the Green/Low category in the traffic light system for MP contamination across all sampling sites in Europe.

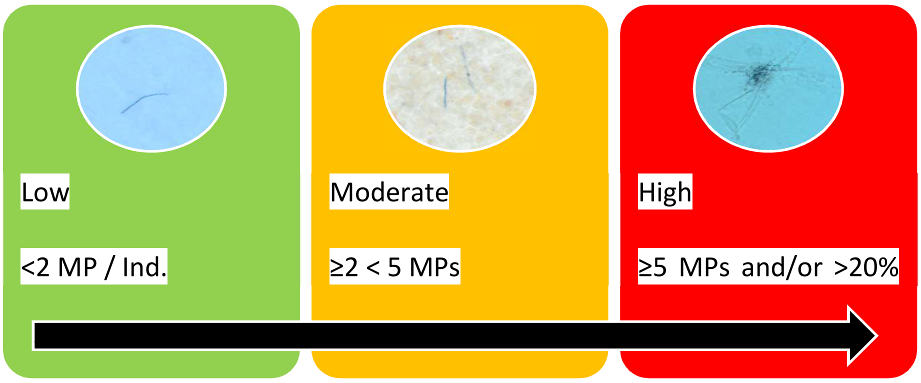

Figure 4. Traffic light system approach to MPs abundances in *Nephrops norvegicus* across Europe.

Example scenarios of traffic light classification:

Green: The Porcupine Bank (FU16) would be classified as having a low MP contamination level from assessing *n*=100 individuals in 2020 (Joyce *et al*. 2022a), 77% had low contamination levels >2 MPs per individual for 75% of the sample, 22% were moderately contaminated having ≥ 2 but < 5 MP particles per individual, and 1% were high having , ≥ 5 MP particles per individual.

Orange: The Aran Prawn Grounds (FU17) would be classified as being moderately contaminated from assessing *n*=100 individuals in 2020 (Joyce *et al*. 2022a), 43% had low contamination levels of >2 MPs per individual, 41% were moderately contaminated having ≥ 2 but < 5 MP particles per individual for > 25% and 16% were high, ≥ 5 MP particles per individual.

Red: The Western Irish Sea (FU15) would be classified as having a high MP contamination level from assessing *n*=100 individuals in 2020 (Joyce *et al*. 2022a), 30% had low contamination levels of >2 MPs per individual, 39% were moderately contaminated having ≥ 2 but < 5 MP particles per individual, and 31% had ≥ 5 MP particles per individual.

4.5 Discussion on the proposed monitoring programme

Based on the research presented here, the proposed monitoring programme would provide information on relative MP loadings in *N. norvegicus* as a potential gateway into the human food chain, and on relative MP abundances in the environment for monitoring purposes. Owing to the knowledge gaps mentioned in section 3.2 *N. norvegicus* has its limitations and further research is needed to address unanswered questions to ensure the proposed monitoring programme is acceptable on a pan-European scale.

For example, sediment sampling and monitoring on a research footing is currently ongoing nationally, and on a pan-European scale, however a consensus on processing SOPs needs to either be agreed, or evolve, to better allow for inter study comparisons. Sediment sampling from a larger depth range of *N. norvegicus* beds will allow for review of MP loadings and comparison to coastal sediments. Furthermore, the MP abundance within the sediments of some of the FUs sampled around Ireland may be expected to be among the lowest levels in Europe (i.e., the Porcupine Bank) owing to their relative proximity to land and open ocean.

The inclusion of a further environmental matrix would provide a more ecosystem-based monitoring approach. Bottom water has been suggested in this regard, however, it is not seen as a logistical or economically feasible medium to sample at present in the North East Atlantic, with some FUs at depths > 500m (e.g., Porcupine Bank). However, the inclusion of bottom water sampling from Mediterranean GSAs and/or shallower coastal embayment's may be a feasible option now or in the future.

Opportunistic sampling may be a more feasible option for both *N. norvegicus* and sediment sampling. A targeted sea going MP monitoring programme would be a sizeable undertaking for relatively small sampling units which may be filled by piggy-backing sampling on other scientific programmes active in the area, therefore it is recommended by the authors to carry out monitoring in conjunction with other sampling efforts as they can offer low-cost opportunities for scientific sampling.

To understand the levels and trends of MP contamination and to draw conclusions, data needs to be comparable from all areas and collated into a larger context on a regional scale to be explored further. One of the main difficulties faced by policy-makers and researchers is the lack of harmonized and standardised methodologies for MPs analysis (Bonanno and Orlando-Bonaca 2018, Kershaw *et al*. 2019). The use of alkaline digestion using potassium hydroxide (10% KOH) has been recommended by many researchers and experts in the field (Bessa *et al*. 2019, Hara *et al*. 2020). The entanglements of fibres in the stomachs of *N. norvegicus* have been proposed as a method for monitoring (Carreras-Colom *et al*. 2022) and observations could be included as an element in the traffic light system approach described here. Other studies carried out on *N. norvegicus* in Sardinian waters found an average of 5.5 ± 0.8 MPs, mainly films and fragments, per positive individual (Cau *et al*. 2019). In a scenario like this, focusing solely on entanglements is insufficient and would lead to the conclusion that low MP levels were present in the environment. Therefore, based on current research the use of entanglements maybe more appropriate in certain geographical regions where they predominate, and potential require only the inclusion of prior visual inspection of entanglements of *N. norvegicus* stomachs to evaluate the level of MP contamination.

Werner *et al*. (2020) stated that to evaluate the status of the environment, the state of a pristine/normal environment is compared to that of an affected one. As a result, reference values must be established against which the existing or potentially changing situation can be evaluated. Under the MSFD Commission decision (EU) 2017/848 (COM DEC), Threshold values should be decided on a European scale. Threshold values relating to MPs have not been developed to date at a national or regional level (Werner *et al*. 2020, DHLGH 2021). Threshold values should be set in relation to "harm" level which includes physical damage, toxicological responses, disruption of human activities and socio-economic damages (Werner *et al*. 2020). However, the threshold concentrations of harm or level of risk to *N. norvegicus* has not yet been established/quantified. Therefore, more information is required to determine threshold values for MP abundance in *N. norvegicus* and benthic sediment in which values can be decided upon (Werner *et al*. 2020). Although adverse effects have been observed, the level of risk is unquantified (Welden and Cowie 2016b) therefore, the threshold values can be established from pristine/near pristine areas. An example of this would be in the case of the OSPAR fulmar bioindicator species using the precautionary principle, which allows for comparisons to be made by utilizing the least polluted area as a reference point (Werner *et al*. 2020). The occurrence of MP particles in the GIT of *N. norvegicus* ideally should be zero, as synthetic materials in the marine environment result from anthropogenic activities and are exogenous to benthic habitats. However, accepting that MP particles are present in the environment, a long-term goal for monitoring MP contamination in *N. norvegicus* is proposed:

“There should be a minimum of 75% of *N. norvegicus* having less than 2 MP particle in their gastrointestinal tract in samples from each of the selected sampling sites across Europe”.

4.7 Conclusion

This paper contributes to D10 of the MSFD in the implementation of a Pan- European MP monitoring programme using *N. norvegicus* as an indicator of MP contamination. Member states are required to establish threshold values for the abundance of micro litter on the seafloor and ingested by marine organism under the MSFD, therefore a long-term monitoring programme is required to determine the trends of MP ingestion for defining these thresholds. Despite *N. norvegicus* and sediment being proposed as a monitoring tool for MP contamination in the benthic environment, the authors recommend a more holistic monitoring approach which would include a variety of species and compartments to be used to cover all ecosystem compartments, for a comprehensive MP monitoring programme, providing a comprehensive database of MP levels and trends in the marine environment. The integrated use of *N. norvegicus* and sediment as a monitoring tool is identified here as being key to monitoring MPs along an extensive spatial scale. In addition, this proposed monitoring programme will compliment current and future MP monitoring, incorporating for instance, fulmars, coastal sediment, and mussels, to allow for a more robust and cost-effective way to monitor MP contamination in the marine environment.

CRediT authorship contribution statement

Haleigh Joyce - Conceptualization, Investigation, Writing - original draft, Fiona Kavanagh- Writing – review & editing, Supervision, João Frias- Writing – review & editing, Supervision, Jonathan White-Conceptualization, Investigation, Writing – review & editing, Alessandro Cau- Writing – review & editing, Ester Carreras-Colom- Writing – review & editing, Róisín Nash-Conceptualization, Writing – review & editing, Supervision.

Declaration of competing interest

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

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5. Discussion and Conclusion

The potential use of different bioindicator species for MP monitoring has been suggested by several researchers across Europe. To explore *N. norvegicus* as potential bioindicator this research first needed to establish a baseline for MP loadings in *N. norvegicus* and in its surrounding benthic sediments. The baseline covered the six primary *N. norvegicus* stocks in the North East Atlantic and showed the ubiquitous nature of MPs within these habitats (Chapter 2). Furthermore, there was no significant relationship between MP abundance in *N. norvegicus*, their biological parameters (sex, size, and moult stage) and their surrounding environment. This would allow for opportunistic samples to be used for monitoring purposes. The MPs abundances, sizes, shapes, and polymer types retrieved in the GIT of *N. norvegicus* also reflected those found in the surrounding sediment samples (Chapter 2). This highlights the potential for *N. norvegicus* to be used for monitoring, demonstrating its capability to reflect the levels of bioavailable MPs in the surrounding environment.

The levels of MPs recovered from the GIT of *N. norvegicus* within the six FU in the North East Atlantic range from low to high (traffic light system - Chapter 4). The average level of MPs recorded per individual from the six primary FU's were low in comparison to other regional studies and showed no evidence of bioaccumulation (Chapter 2). Distance from shore and proximity to highly industrialised areas and/or densely populated areas might contribute to concentrations of MP loadings e.g., Porcupine Bank and Western Irish Sea. These variations support the ability of *N. norvegicus* to reflect local levels of MP in the surrounding environment and to detect the variation in MP availability between locations. With the ever-increasing production of plastic and therefore MPs in the marine environment, the monitoring of MP contamination is vital to ensure MP levels are either stable or do not increase.

The authors acknowledge that while entanglements of MPs were recorded within the stomachs of organisms in areas of low MP contamination, these were not a prominent feature in this study. While the majority of fibres retrieved from *N. norvegicus* in the North East Atlantic were MPs (i.e., <5 mm - 97.4%) (chapter 2) those > 5mm (i.e., mesoplastics) appeared to be retained when examined in the retention study (Chapter 3) illustrating that these may be retained for longer before being egested from *N. norvegicus*. The fibres of greater lengths may not align with the gastric mill and therefore, would not be passed through the hindgut to be excreted. Fibres (>5 mm) may then become twisted or entangled due to the churning movement of the stomach during digestion causing potential adverse effects. These entanglements of fibres have been previously reported in *N. norvegicus* and other marine biota (Hara *et al*. 2020, Craig *et al*. 2022) and have shown to be more prominent in areas of high MP ingestion in *N. norvegicus* (Carreras-Colom *et al*. 2022). Therefore, there is the potentially for organism to give a representation of the levels of bioavailable MPs.

The proposed pan-European monitoring programme integrates the use of *N. norvegicus* and sediment as a monitoring tool to identify spatio-temporal trends of MP contamination on a European scale (Chapter 4). The outlined monitoring programme presented here is based on investigative studies (Chapter 2 and 3), relevant literature and input from researchers and policy makers across Europe. The monitoring programme is a combination of *N. norvegicus* with benthic sediment to provide a better understanding of microplastic accumulation and distribution in the North Atlantic and in the Mediterranean Sea.

Consideration has been given to the feasibility and costs effectiveness of the monitoring programme; therefore, an opportunistic approach has been taken, in relation to sampling, which will be carried out in conjunction with current established surveys where possible. The pilot study will ensure an agreed level of spatial and temporal coverage and precision required to carry out the monitoring programme. This research, and more specifically the Pan- European MP monitoring programme, aligns with the requirements of D10 of the Marine Strategy Framework Directive (MSFD) using *N. norvegicus* and sediment as a monitoring tool.

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