Marine Pollution Bulletin 152 (2020) 110905

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Quantification of microplastic ingestion by the decapod crustacean *Nephrops norvegicus* from Irish waters

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ARTICLE INFO

Keywords:
Biomonitoring
Seafood
Contamination
Food safety
FTIR

ABSTRACT

Microplastics are widespread pollutants in the marine environment, yet few studies have assessed the abundance and characteristics of microplastics in commercial species. This study evaluates the presence of ingested microplastics in the gastrointestinal tract of *Nephrops norvegicus* ($n = 150$), collected from five Irish prawn grounds. The efficiency of three digesting solutions was assessed. The most efficient digestion was the KOH (10%) solution incubated at 40 °C for a 48 h period. An average of 1.75 ± 2.01 items per individual was ingested by *c.* 69% of *N. norvegicus* examined. A total of 262 microplastic, predominantly fibres (98%), between 1 and 2 mm were recorded. Although, no spatial pattern was identified, samples from the North Irish Sea recorded highest occurrence of microplastics (~83%). A positive correlation was found between microplastic abundance and prawn carapace condition. Results indicate microplastic exposure in seafood for human consumption, in Ireland, is estimated to range from 15 to 4471 particles per year.

1. Introduction

Microplastics (MPs), are synthetic materials with a defined size ranging between 1 μm to 5 mm (Frias and Nash, 2019), and are considered ubiquitous pollutants in the marine environment (Lusher et al., 2013; Browne et al., 2007). The widespread contamination of microplastics is a growing worldwide problem (Cole et al., 2011), as microplastic particles have been found in the open ocean (Cózar et al., 2014), coastlines (Nel and Froneman, 2015), inshore/offshore sediments (Alomar et al., 2016; Reddy et al., 2006), shelves and deep-sea basins (Pham et al., 2014), posing potential environmental risks to a diverse range of marine organisms, mainly through ingestion (Wright et al., 2013; Lusher, 2015).

Current microplastics trends in the environment, suggest that most groups of marine organisms, such as detritivores (amphipods *Orchestia gammarellus*), deposit feeders (lugworms *Arenicola marina*), and filter feeders (barnacles *Semibalanus balanoides*) (Thompson et al., 2004; Wright et al., 2013), including crustaceans, are under threat (Do Sul and Costa, 2014).

Despite these concerns, and taking into consideration that only a few numbers of studies specifically examine the presence of MPs in natural populations (Devriese et al., 2015), studies that assess baseline levels of microplastic contamination are still lacking for many species and regions worldwide (Lusher et al., 2017a, 2017b; Karlsson et al., 2017), mainly commercial species.

The European Food Safety Authority (EFSA) recently emphasized the need for establishing MP occurrence data in relevant species, with a particular focus on seafood products (EFSA-CONTAM, 2016). As a result, MP contamination in seafood and its potential consequences are currently becoming a major interest, as it has raised concerns related to food safety (Van Cauwenberghe and Janssen, 2014; Karami et al., 2017a, 2017b), requiring mandatory and priority attention from concerned stakeholders, including researchers, decision makers, and the general population (Costa et al., 2018; Smith et al., 2018).

Nephrops norvegicus (Linnaeus, 1758) commonly known as Dublin Bay Prawn or as Norway Lobster, is considered the most important commercially crustacean in Europe (Bell et al., 2006; Bell et al., 2013). This benthic burrowing species inhabits muddy bottoms of the North-Eastern Atlantic and the Mediterranean, between 20 and 800 m depth (Welden et al., 2015; Bell, 2015). FAO (2010) reported that landings of this species steadily increased since 1950s, with currently having an estimated total catch exceeding 55,000 tonnes annually (Bell et al., 2006). According to Ireland's Seafood Development Agency (BIM, 2017), it is an extremely valuable species in the country as it supports an important fishing industry, with landing statistics in recent years estimated to be *c.* 7800 tonnes, representing a significant part (11.7%) of the total landings in European waters (Ungfors et al., 2013).

Despite research conducted in recent years in this field, there is no

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https://doi.org/10.1016/j.marpolbul.2020.110905
Received 17 October 2019; Received in revised form 9 January 2020; Accepted 9 January 2020
Available online 15 January 2020
0025-326X/

Fig. 1. Five prawn sampling grounds: Aran Prawn Ground (APG), Magharees Union (MU), Kenmare Bay (KB), Bantry Bay (BB), and the North Irish Sea (NIS). Map adapted from the Ireland's Marine Atlas (Irish Marine Institute Ireland's Marine Atlas, 2016).

defined protocol for the extraction of ingested microplastics. To date, the detection and quantification of microplastics in biological samples has been carried out using several techniques (Silva et al., 2018), including both acids (Devriese et al., 2015; De Witte et al., 2014) and bases (Claessens et al., 2013; Foekema et al., 2013) as digesting agents. An accurate evaluation of MP ingestion using standardized methods is crucial and still remains a challenge (Mai et al., 2018), thus emphasizing the need for a standardized protocol which takes into account target organisms and applicable test solutions. Silva et al. (2018) and Lusher et al. (2017a, 2017b) emphasized that the use of validated analytical techniques is vital for quality control as well as comparability of results between studies. There is also the concern that unsuitable protocols could potentially result in the underestimation of the abundance of microplastics present and some of the digesting agents applied could affect the integrity of the plastic polymers, particularly when used in combination with high temperatures (Karami et al., 2017a, 2017b; Dehaut et al., 2016).

In reviewing relevant literature, the authors noted that the protocols used to quantify microplastics in biota have varied greatly between studies (Li et al., 2015; De Witte et al., 2014; Devriese et al., 2015; Foekema et al., 2013). In addition, there are few studies on commercial species of decapod crustaceans, the most notable being the study conducted by Murray and Cowie (2011), and the previous work quantifying the plastic ingestion of the brown shrimp *Crangon crangon* (Devriese et al., 2015). Though there are reports that demonstrated MP contamination in wild *N. norvegicus* populations in Scotland (Murray and Cowie, 2011), to the authors' knowledge a definitive prevalence record of microplastic ingestion in populations within Irish waters has not yet been identified particularly across a variety of known prawn grounds. Martin et al. (2017) reported that the fishery stocks may be at high risk of exposure to MP contamination.

Recent research efforts that identified potential dietary exposure from MP have been also conducted for other commercially important species such as the blue mussel, *Mytilus edulis* (Van Cauwenberghe and Janssen, 2014) and dried fish, such as Indian mackerel (*Rastrelliger kanagurta*), spotty-face anchovy, (*Stolephorus waitei*), greenback mullet (*Chelon subviridis*), and belanger's croaker (*Johnius belangerii*) (Karami et al., 2017a, 2017b). These studies represent an increasing scientific evidence that various seafood products contained plastic particles, and that the potential consequences related to food safety need to be considered. Evidence on the abundance of microplastic particles in these products and associated exposure to consumers have, so far, been limited (Barboza et al., 2018), therefore further information is required which could serve a basis to communicate any possible risks and implement appropriate measures, if necessary (Lusher et al., 2017a, 2017b).

As such, the primary aim of this study is to evaluate the prevalence of microplastic contamination in the gastrointestinal tract of *Nephrops norvegicus* collected from different commercial prawn grounds on the west and northeast coast of Ireland. The authors hypothesize that there is variation in the rate of microplastic contamination within the natural populations of *N. norvegicus* in Irish waters. Specifically, this study aimed to: (i) verify the most efficient and effective MP extraction methods from the gut of *N. norvegicus*; (ii) establish whether *N. norvegicus* collected from commercially fished prawn grounds had ingested microplastics; (iii) identify the characteristics of microplastic particles present through physical and polymer characterization; (iv) assess whether there were differences, in the quantity and characteristics of ingested microplastics among different sources, sizes, and moulting stages; and (v) determine the potential exposure to microplastics from the consumption of seafood *N. norvegicus*.

2. Materials and methods

2.1. Study area

Wild prawn populations of *Nephrops norvegicus* were sampled from five commercially fished prawn grounds around the west and northeast coast of Ireland in distinct sandy/muddy areas; namely: (i) the Aran prawn ground (APG), (ii) Magharees Union (MU), (iii) Kenmare Bay

(KB), (iv) Bantry Bay (BB), and the (v) North Irish Sea (NIS), as shown in Fig. 1. These areas are defined as *Nephrops* grounds by the Irish Marine Institute as a result of their Under-Water Television Surveys (UWTV).

The *Nephrops* stocks covered in this study are part of three functional units (FUs 15, 17 and 19) of the ICES assessment area within the Irish Coast (ICES, 2018). Trawling is the primary means of *Nephrops* fishing within these grounds (Ungfors et al., 2013), with minimum cod-end mesh size from 70 to 80 mm (BIM, 2015; ICES, 2018). These grounds are considered to have a moderate to high burrow densities, with an estimated value of 0.5–0.9 burrows per m^2 (Ungfors et al., 2013).

2.2. Collection of samples

All samples used in this study were provided by the Irish Marine Institute and were obtained from scientific surveys and commercial fisheries between November–December 2016. Sample collection was carried out using beam trawls within the five pre-established prawn grounds sampling locations, by the RV *Celtic Voyager*. A 4 m beam trawl with a mesh size of 80 mm in the cod-end was trawled for approximately 30 min at a speed of 4 knots over a distance of 4 nautical miles. The specified gear is similar to the one used by the fishing industry, and the collected organisms were then representative of those caught for commercial markets.

Following the recovery of each trawl onto the deck, the *N. norvegicus* samples were identified, sexed and frozen for preservation.

2.3. Laboratory analysis

2.3.1. Extraction methods: digestion efficiency and polymer recovery rates

The efficiency and effectiveness of existing protocols intended for MP extraction from biota, such as suggested by Karami et al. (2017a, 2017b) for fish, were tested for the digestion of biological materials contained in the gastrointestinal tract of *Nephrops norvegicus*. Here the gastrointestinal tract of *N. norvegicus* is understood to consist of the stomach – which is separated into the cardiac (CS) and pyloric foregut (PS), the mid gut and hind gut, according to Welden et al. (2015).

The digestion methods and prevalence of plastic polymers were evaluated through determining the digestion efficiency and plastic recovery rates using different digesting solutions under controlled conditions, with defined amount and typologies of plastic polymers spiked in the gut samples. Three digesting solutions (Potassium Hydroxide (KOH, 10%), KOH with Tween20 (KOH + T20, 10%) and Nitric Acid (HNO3, 69%) were tested in triplicate at different temperatures (40 °C, 50 °C, and 60 °C) for 24 to 48 h to assess the best method of extraction of six types of microplastic polymers (Polypropylene (PP), Polystyrene (PS), Polyethylene Terephthalate (PETE/PET), Polyamide (PA 6,6), High-Density Polyethylene (HDPE), and Low-Density Polyethylene (LDPE)). Tween20, is a polysorbate-type non-ionic surfactant that can be used in cell lysing when combined with KOH (Xia et al., 2019). Visual examination and manual shaking of the digestates were performed during the incubation period.

Each of the six plastic polymers to be tested were stained with Nile Red (NR) solution to provide an effective and convenient means of identifying reference microplastics in controlled samples mixed with inorganic materials in laboratory experiments (Shim et al., 2016). Staining was carried out by adding 1.5 mL of Nile Red stock solution in acetone (CH_3COCH_3) to the plastic polymers in 2.5 mL Eppendorf tubes (Maes et al., 2017). The solutions were then centrifuged at 5*g* for 1 min and allowed to rest for approximately 5 h in a fume hood until remaining solution evaporated. Spiked polymers were weighted, counted, and photographed before and after application of the digestion protocol to determine potentially surface damaging effects to plastics. Reference stained polymers were preserved and used for surface topography and colour comparison, while procedural blanks for all digesting solutions were prepared and performed simultaneously for each treatment.

After the digestion process, the digestates were then filtered using a vacuum pump (VWR™ VCP 130) through a 47 mm Whatman® GF/C glass microfiber filter membrane. Before and after filtration, all filter membranes were maintained at 40 °C for 5 h and weighed using an analytical balance (Adventurer™ Ohaus AR2140).

The rates of digestion efficiency and polymer recovery were determined following the formula below adopted from Karami et al. (2017a, 2017b):

$$\textit{Digestion Efficiency}\ (\%) = \frac{Wi - (Wa - Wb)}{Wi} \times 100$$

where Wi = Initial weight of biological materials and spiked polymers; Wa = Weight of dry filter membrane after filtration; Wb = Weight of filter membrane before filtration.

$$\textit{Polymer Recovery}\ (\%) = \frac{Wa - Wb}{Wi} \times 100$$

where Wa = weight of the filter membrane after filtration; Wb = weight of the filter membrane before filtration; and Wi = initial weight of the spiked MPs.

2.3.2. Morphometric measurements and carapace condition

Prior to dissection, samples were defrosted and morphometric observation and measurements including the sex, carapace length, carapace hardness and physical damage for each individual were recorded. Carapace length was measured from the eye socket to the base of the carapace using digital callipers (Moore&Wright™). Carapace condition was determined following the protocol developed by Milligan et al. (2009), and used as a simple measure of the moult stage of each individual sample. Based on the classification categories of (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". The moult stage was then determined based on the carapace condition, in which hard animals were assumed to be at intermoult stage; jelly animals were assumed to have moulted very recently and soft animals assumed to be either at late intermoult with removed calcium from the exoskeleton or recently moulted stage but no longer jelly (Milligan et al., 2009; Murray and Cowie, 2011).

Physical damage on the external structure and body parts such as claws, limbs, eyes, and soft tissue were also determined based on the category and damage index introduced by Ridgway et al. (2006), with three different categories such as the following (a) no damage, (b) slight damage and (c) severe damage.

2.3.3. Microplastics analysis

Digestive tracts, consisting of foregut and midgut, were removed through the dissection of each prawn and then immediately transferred to a decontaminated glass jar. The extraction of microplastics was performed based on the results of the preliminary experiment of different protocols, as previously mentioned.

The resulting digestate was filtered using a vacuum pump through a 47 mm Whatman® glass microfiber filter paper. The filter was then transferred onto a labelled petri dish for visual examination and sorting of microplastics was performed under a stereo microscope (Micros Austria Hornet Micro Zoom 1280).

Physical characterization of the observed microplastics through visual assessment was performed using the Olympus SZX10 microscope and Image Pro-Plus software. The extracted microplastics were counted, photographed and measured through an ocular micrometre and categorized depending on size, colour and shape/type (fibre, fragment, film, etc.).

Polymer identification of larger microplastics was carried out using a Perkin Elmer Spectrum Two FT-IR Spectrometer equipped with ATR-FTIR module, and identification of smaller microplastics was carried

out using a Bruker Hyperion 2000 FT-IR Microscope with a MCT (mercury-cadmium-telluride) detector. All matches with reference database were above 80%.

For the Perkin Elmer Spectrometer, the simultaneous measurement of representative samples of microplastic particles was performed in transmission mode in a wave number range of 4000–400 cm^{-1} using a spectral resolution of 4 cm^{-1}. A total number of eighteen scans were set for every spectrum taken for each sample. The background spectrum was measured with the same parameters before scanning the microplastic samples.

For the Bruker FT-IR microscope, samples spectra were collected in transmission mode in 64 scans, with a spectral resolution of 4 cm^{-1}, in a wavenumber range of 4000–400 cm^{-1}. As previously mentioned, background spectra were measured with same parameters prior to scanning the microplastic samples.

2.3.4. Contamination control

Strict measures were carried out while handling and processing the samples to mitigate contamination and cross-contamination of airborne and solvent microplastic particles. As such, 100% cotton laboratory coat and nitrile gloves were worn at all times. All tools, equipment and work surfaces were thoroughly cleaned prior to use, and throughout the experiments mainly non-plastic tools (metal and glass) were used. All glassware was decontaminated in a dilute Nitric Acid (HNO_3) (0.05%) wash, followed by rinsing with ultrapure water. The working station was subjected to contamination controls, wherein a clean filter paper was used to sample and assess any airborne synthetic fibres during the whole process of the laboratory analyses. To ensure quality assurance and other potential contamination, procedural blanks using ultrapure water and digesting solutions (KOH) were evaluated simultaneously during digestion processes.

2.4. Data analysis

The rates of digestion efficiency and polymer recovery among treatments, as well as the levels of microplastic ingestion from the five prawn grounds in Ireland were compared to determine whether there are any statistically significant differences using parametric analysis by one-way ANOVA (multiple groups). A correlation analysis (Spearman Rank Correlation) was performed to examine the relationship between the abundance of microplastic and the physical characteristics (body weight, carapace length and condition) of the tested samples. Statistical analyses were carried out using RStudio version 1.2.

3. Results

A total of 150 *Nephrops norvegicus*, 30 from each prawn ground, all of which were identified as males, were used to determine the abundance and characteristics of ingested MPs around the Irish coast, while 36 *N. norvegicus* from the North Sea prawn ground were used to determine the optimal digestion method to apply.

3.1. Digestion efficiency

No significant statistical differences in the mean rate of digestion efficiency between treatment groups ($F_{8,18} = 5.001$, $p > 0.001$). However, the digestion using KOH (10%) solution incubated at 40 °C for 48 h was slightly more efficient when compared to the other treatments, with an average percentage of 96.03 ± 2.15 (Fig. 2). Similarly, the combination of KOH and Tween20, at 50 °C resulted in a high efficiency rate that was within the optimum range set between 95%–105%. In comparison, incubating the gut contents with HNO_3 solution at any of the temperatures within the 48 h period resulted in an average digestion rate of > 110%, which led to a complete digestion of biological materials, but also the degradation of some plastic polymers, particularly Polyamide (PA, 66).

Observations recorded during each of the steps (digestion, filtration and filter observations) towards the development of an optimal methodology are available in Table 1. Visual examination under the microscope revealed the presence or absence of residue on the filter membranes. Through analysing the filters, the author determined that where a negligible amount or absence of residue (filtrates/debris) was recorded it allowed for a higher detection and subsequent sorting of microplastics. A foam was observed during digestion for those treatments incubated at the higher temperatures of 50 °C and 60 °C, and particularly for the treatment using KOH + T20. This foam contributed to the clogging of the filters and subsequently to a reduction in filtration rates.

Digestion efficiency, as referred to in the first step in Table 1, includes how effective the digestion of the gut lining was. Where it was evident that no biological material remained this signified an effective and satisfactory digestion efficacy of the solution (Fig. 3b) as opposed to undigested gut lining apparent in Fig. 3a.

3.2. Polymer recovery

To assess the different protocols in terms of recoverability, samples were spiked with six different plastic polymers including LDPE HDPE, PETE, PS, PP and PA. No significant differences were observed in the recovery rates of the spiked polymers among the treatments ($F_{8,18} = 5.415$, $p > 0.001$). While there was no significant difference in the recovery rate the author also observed no modification of the physical structure, weight, and shape of polymers after 48 h of digestion using KOH and KOH + Tween20 solutions, with high recovery rates (%) ranging from 99.88 ± 0.15–102.74 ± 2.07% and 99.80 ± 0.12–101.00 ± 0.66%, respectively (Fig. 4).

However, the concentrated HNO_3 caused the degradation of some polymers resulting in lower recovery rates < 96%, particularly at 40 °C and 50 °C (Fig. 4). It was also observed, that at 60 °C, most of the polymers had partially melted and deformed, resulting in a recovery rate higher than its initial weight (117.81 ± 13.68%). The only plastic polymer, which was consistently degraded at all temperatures, where concentrated HNO_3 was used as the digesting solution was Polyamide 6,6. After microscopic examination and in comparison, with the Nile-red stained reference polymers, all other polymers were observed to have some defects in the structure and changes in colour, e.g. removal of the nile red stain and alterations in colour (yellowing) of all other polymer types as is shown in Fig. 5.

3.3. Microplastic ingestion

A total of 262 microplastic particles were extracted from the digestive tracts of *N. norvegicus*, with an average of 1.75 ± 2.01 items per individual. Of these samples, 103 out of 150 individuals (*c.* 69%) had ingested at least 1 MP particle. The minimum fibre length recorded was 143.20 μm.

No significant differences were observed in the level of MP ingestion between prawn grounds sampled ($F_{4,145} = 2.389$, $p > 0.05$). However, samples collected from Kenmare Bay (KB) exhibited the highest MP abundance among the five prawn grounds with an average of 2.30 ± 2.47 items/individual, while the lowest was recorded in Aran Prawn Grounds (APG) (0.90 ± 1.03 items/ind) (Fig. 6). Strict control measures were employed to prevent contamination of airborne microplastic particles during the laboratory analysis, wherein results revealed that the procedural blanks and air control only contained 0.14 ± 0.38 items/filter and 0.38 ± 0.55 items/filter, respectively.

The percentage of microplastic occurrence for each of the sampling sites is presented in Table 2, where samples from the North Irish Sea (NIS) recorded the highest proportion of individuals positive for MPs ingestion (83.33%), while the lowest recorded is 56.67% for Aran Prawn Grounds (APG). The abundance of microplastic particles ranged from 1 to 10 items per individual, depending on the sampling locations.

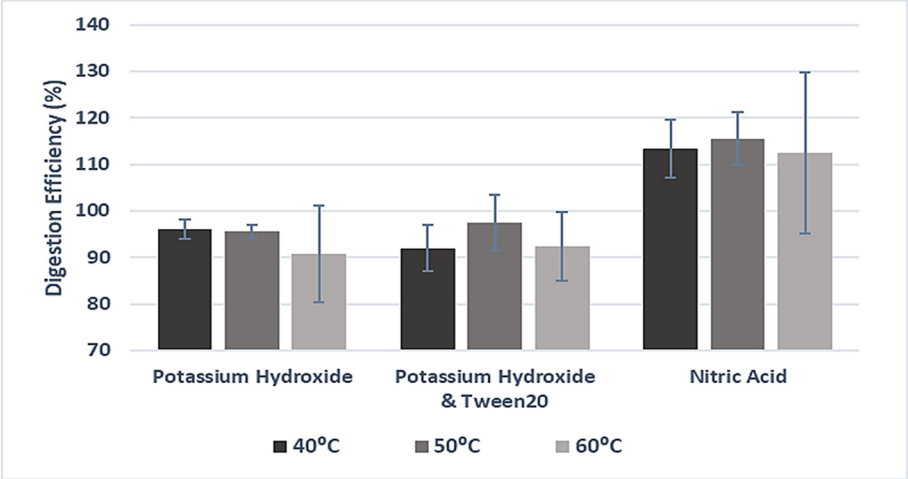

Fig. 2. Digestion efficiency rate (%) of biological materials from the gut of *Nephrops norvegicus* ($N = 36$) incubated in digesting solutions across different temperatures (40 °C, 50 °C and 60 °C).

Table 1
Quality of the digestion, filtration and filter observations after digestion of the gut of *Nephrops norvegicus* using digesting solutions: Potassium Hydroxide (KOH, 10%), KOH with Tween20 (KOH + T20, 10%) and Nitric Acid (HNO3, 69%).

Steps	Protocols		
	Potassium hydroxide	Potassium hydroxide and Tween20	Nitric acid
Digestion	Gut lining not fully digested. Small amount of foamy particles and residue.	Gut lining not fully digested. Dense digestates due presence of foamy particles and residue.	Full digestion within 24 h. Clear digestates, with no particles visible to the naked eye.
Filtration	One to three filters used, depending on the individuals.	Maximum of five filters used, depending on the individuals. Continuous clogging was an issue in some samples.	Fast filtration, no clogging issues.
Filter examination	Negligible amount of residue.	Residue present.	No residue.

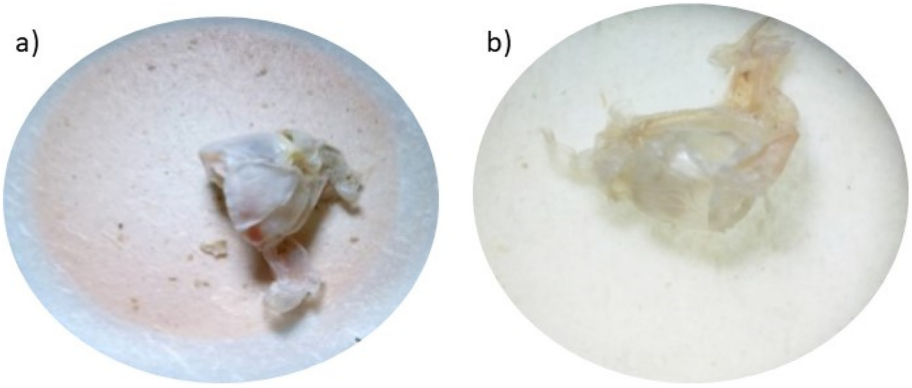

Fig. 3. Gut of the *N. norvegicus* after the application of the digestion protocol using Potassium Hydroxide (KOH) at 40 °C: a). Filter membrane with the undigested gut lining and b) Filter membrane with the digested gut lining.

3.4. Particle composition

The majority of the ingested microplastics found in this study were categorized as fibres (98.1%), followed by fragments (1.5%) and films (0.4%). The ingested fibres recorded ranged from 0.143 to 16.976 mm, having an average length of 2.81 mm. The most common size class recorded is within the range of 1 to 2 mm (32.0%). Results showed that 86.2% of all the extracted particles were within the defined size for microplastics (> 1 μm and < 5 mm), while the rest, consisting of particles with size larger than 5 mm represents *c.* 13.8%, highlighting the occurrence of macroplastics among the extracted particles. Fig. 7(a–f) shows the microscopic images of some of the extracted plastic particles.

Entangled fibres (Fig. 7b) were commonly extracted in gut samples from Kenmare Bay, while the fragments and films (Fig. 7a & c) were both found in samples from the North Irish Sea, which also contained the most different microplastic types confirmed within a single station ($n = 3$).

Fig. 8 presents a stacked bar chart showing the colour composition of the extracted particles for each of the prawn grounds. In general, the ingested microplastics were represented by a variety of colours with blue (48.1%) being the most prevalent, followed by black (32.8%), grey (11.8%), red (6.1%), green (0.8%) and multicolor (0.4%).

3.5. Polymer identification

A subset of extracted microplastic particles were selected and

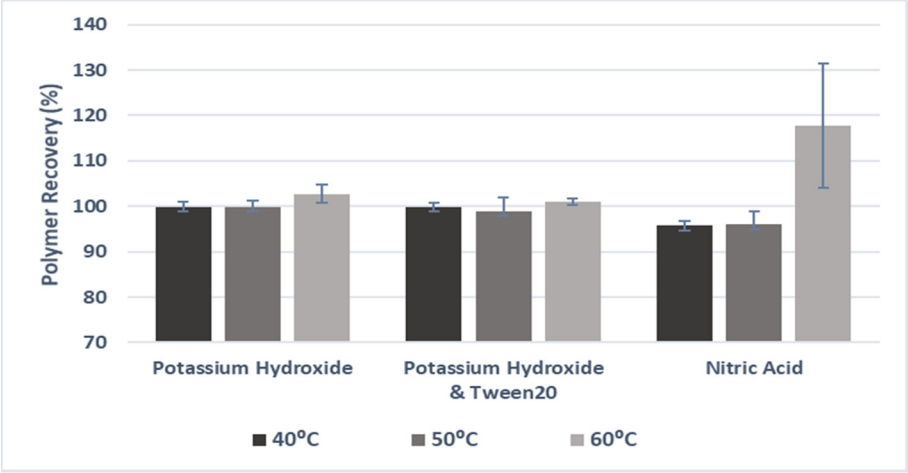

Fig. 4. Recovery rate (%) of plastic polymers ($N = 18$) spiked in biological materials from the gut of *Nephrops* and incubated with digesting solutions across different temperatures (40 °C, 50 °C and 60 °C).

analysed for polymer identification. The main characteristic bands of the spectra for larger particles representing the different microplastic types (fragment, entangled fibre, and film as shown in Fig. 7) are listed in Table 3, together with its assignment and vibration type as per reference values available in the literature (Shashoua, 2008). Based on the characteristic bands and peaks of the spectra obtained through FTIR analyses, these three microplastic particles were composed of polyvinyl chloride (PVC), polyamide (PA), and polyethylene (PE), with match percentages approximately of *c.* 92%, *c.* 80%, and *c.* 84%, respectively. For the subset of the fourth type of extracted microplastics (fibres), FTIR results revealed that it is mainly composed of polystyrene, polypropylene, polyester, polycarbonate, and polyethylene, while some were specifically identified as components of windscreen wiper and sealing ring. The remaining microplastic fibres, which were not analysed for polymer identification was accepted solely through a physical characterization and comparison with the identified particles.

Similar characteristic bands of sample and reference spectra confirmed the occurrence of synthetic particles in the gastrointestinal tract of *N. norvegicus*. Among the particles confirmed as microplastics, the prevalence of the confirmed PVC and PE polymers were only recorded in individuals sampled from the NIS. While the concentration of entangled fibres confirmed using a subset sample as PA polymer was only extracted in samples from KB. As there is a similar stretching, band peaks for all samples particularly within the range of 3500–3000 cm^{-1} and 1000–400 cm^{-1}, possibly due to the characteristic vibration of OH. This assumption was verified and compared to any available literature related to tissue digestion using Potassium Hydroxide as (KOH) as digesting solution.

3.6. Patterns of variation in microplastics ingestion

As no significant differences in microplastic contamination between areas were demonstrated, data from the different areas were combined to analyse patterns of ingestion depending on physical characteristics such as body weight, carapace length and condition. Carapace length (CL) ranged between 24.4 and 48.0 mm, with an average size of 36.15 ± 5.58 mm. Out of the 150 samples measured, approximately 77.3% were found to have a CL within the estimated size at onset of sexual maturity (SOM), (29–46 mm.) for male *N. norvegicus* (Tuck et al., 2000). Mean microplastic count as per carapace size ranges is shown in Fig. 9. Mean frequency distribution of ingested microplastics in relation to carapace length, wherein the highest average count of ingested microplastics was recorded in individuals with carapace length within the range of 46–50 mm. A Spearman's correlation was run to assess the relationship between the rate of microplastic ingestion and carapace length, based on 103 complete observations for individual samples positive for ingestion, with non-missing values for both variables. There was a moderately positive correlation between the rate of microplastic ingestion and carapace length, which was statistically significant at the 0.05 level (2-tailed), rs = 0.237, $p = 0.016$.

Fig. 10 shows the proportion of the carapace condition such as hard and soft representing the moult stage of each individual, distributed along with the level of microplastics ingestion. Most of the tested individuals (*c.* 63%) were observed to have hard carapace condition, which is assumed to be at the intermoult stage. No jelly individuals were recorded, while animals with soft carapace condition represent 37.3% of the sample. A high rate of microplastics ingestion having 8–10 items/individuals were recorded in samples assumed to be at intermoult stage (hard carapace) and all within the estimated size at onset of maturity. However, results for correlation analysis indicated no significant association ($p > 0.05$) could be found between microplastic ingestion rate and carapace condition of the tested samples (rs = 0.013, $p = 0.892$).

The average wet body weight, excluding the claws, is 23.44 ± 10.67 g, with a maximum and minimum values equivalent to 61.3 g and 7.1 g, respectively. Using the three-level index and criteria introduced by Ridgway et al. (2006) to categorize the extent of damage, results show that a large proportion of dissected *N. norvegicus* has severe damage (68%) on its external structure, mainly exhibiting loss of some body parts such as claws. Only 6% of the total samples have been categorized with no damage, while 26% as slightly damage. Similarly, a moderately positive significant correlation ($p < 0.05$) could be found between microplastic ingestion and body weight of the tested samples (rs = 0.244, $p = 0.013$).

3.7. Microplastics exposure from the consumption of N. norvegicus

The MP exposure from the consumption of *N. norvegicus* was calculated based on different MP concentration and seafood consumption scenarios (Table 4). Estimations were made by assuming that the tested samples represent the EU Council Regulation (EC 2406/96) marketing standard size grade III (121–180) count per kilogram of tailed prawns and using the estimated dietary intake (7 g/day) of crustaceans for adult consumers (18–64 years old) in Ireland (EFSA, 2014).

Different scenarios of MP concentration for individual prawn were established using the average concentration in this study, namely the worst-case scenario having 1.75 particles for individuals consumed with intact gastrointestinal tract, while good to best case scenarios that 50% (0.875 particles/ind.) and 90% (0.175 particles/ind.) of the ingested microplastics will be removed by peeling. Based on the results of

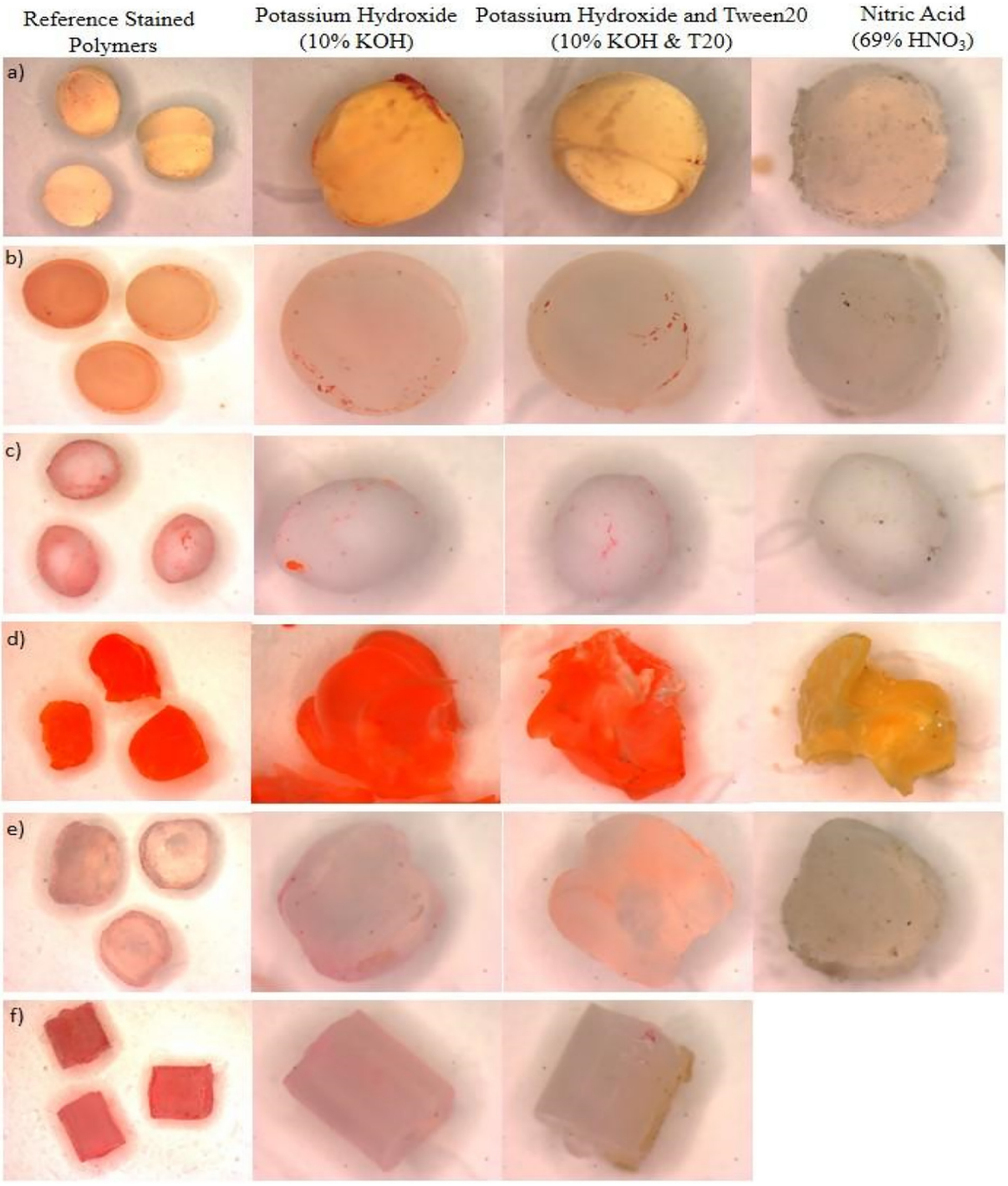

Fig. 5. Microscopic observation of reference and spiked plastic polymers incubated with digesting solutions across different temperatures: a) Low-density Polyethylene (LDPE), b) High-density Polyethylene (HDPE), c) Polyethylene Terephthalate (PETE), d) Polystyrene (PS), e) Polypropylene (PP) and f) Polyamide (PA 6,6). No image was taken for PA as it was fully degraded after using HNO_3.

the estimated dietary exposure to microplastics given the three different consumption scenarios (daily, weekly, monthly) for prawns with intact gastrointestinal tract, the MP exposure from the consumption of prawns of a reference Irish adult consumer is expected to be between 147 and 4471 MPs/year. While, an estimated dietary microplastics exposure within the range of 15–447 MPs/year are still expected for consumers of prawns with removed gastrointestinal tract (90%).

4. Discussion

4.1. Extraction methods for ingested microplastics in N. norvegicus

Developing, validating and recommending a harmonized or standardized protocol to determine the digestion efficiency for extracting the ingested MPs is, as stated by Silva et al. (2018), crucial for the quantification of microplastics. However, studies related to plastic ingestion of decapod crustaceans are still limited (Murray and Cowie, 2011). Earlier studies conducted on plastic ingestion of decapod

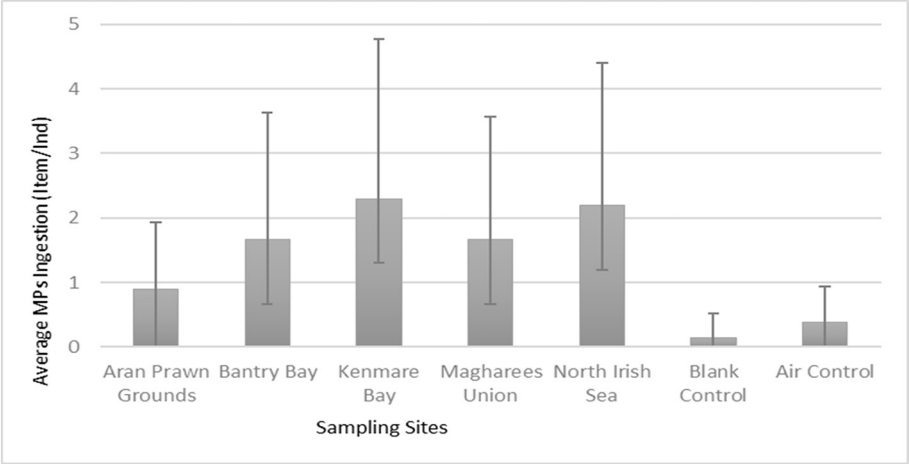

Fig. 6. Average (± SD) microplastics observed in *N. norvegicus* sampled from the five different prawn grounds (N = 150).

Table 2
Variation in microplastic occurrence and abundance at each of the prawn grounds and the proportion of individuals at each site (N = 150) that recorded microplastics.

Sampling stations (prawn grounds)	Total MPs recorded	Maximum MP count	Percentage containing MPs
Aran Prawn Grounds (APG)	27	4	56.7
Bantry Bay (BB)	50	10	73.3
Kenmare Bay (KB)	69	10	70.0
Magharees Union (MU)	50	7	60.0
North Irish Sea (NIS)	66	9	83.3

crustaceans in Europe mainly included species such as *Nephrops norvegicus* and *Crangon crangon*, where MPs are extracted through visual examination under a microscope (Murray and Cowie, 2011; Welden and Cowie, 2016a, 2016b) or acid digestion (Devriese et al., 2015) of the stomach content.

The authors agree with both Avio et al. (2015) and Lusher et al. (2017a, 2017b) that a digestion step is crucial for extracting MPs from biota. Eliminating biological materials and tissues that might mask synthetic particles could contribute to the underestimation of microplastic abundance recorded. Karami et al. (2017a, 2017b) reiterated the fact that an efficient digestion process without compromising the integrity of plastic polymers is required, while suggesting a high-performance protocol intended for the extraction of MPs in fish samples. This protocol can be adapted to digest biological materials such as digestive contents of other marine organisms.

Results confirmed that KOH (10%) incubated at 40^{0}C is the most efficient solution for digesting gut tissues of *N. norvegicus*, comparable to the results of Karami et al. (2017a, 2017b), demonstrating the highest digestion efficiency rate among treatments. As observed, digesting solutions incubated at 60 °C is the least efficient among the three, which shows that an increase in the temperature could affect the polymer. Patil and Sharma (2011) described that the specific reaction of KOH solution varies depending on temperature. Dehaut et al. (2016) employed the same protocol with higher incubation temperature (60 °C) exhibiting efficient digestion of biological tissues without any significant degradation on spiked polymers, applied on other seafood products such as crabs, fish, and mussels. Similarly, Rochman et al. (2015) reported the applicability of a similar protocol to quantify the presence of anthropogenic debris in digestive tracts of fish and bivalves

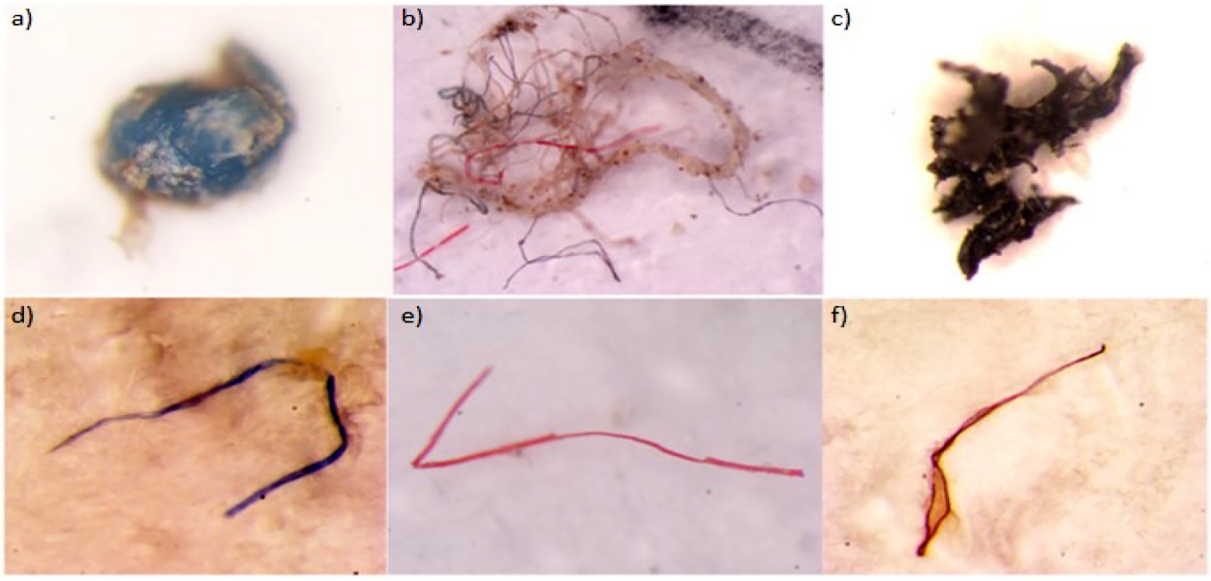

Fig. 7. Images of different types of microplastics extracted from the gut of *N. norvegicus*: a) blue fragment; b) entangled fibre, c) black film, and d–f) blue, red and black fibres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

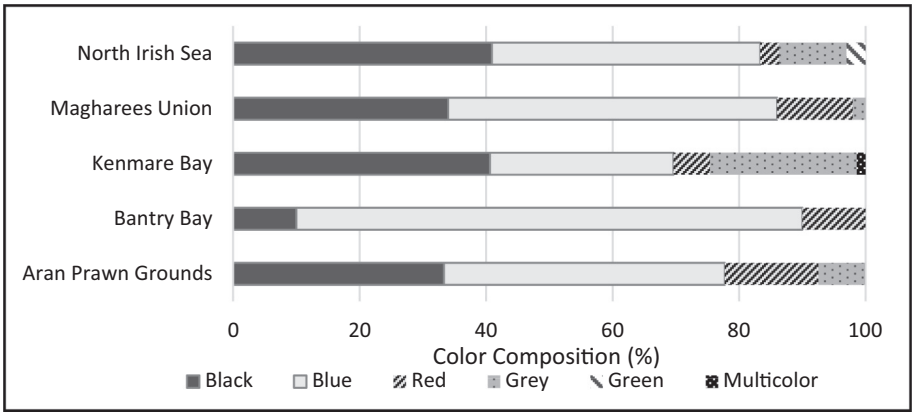

Fig. 8. Colour composition of the extracted microplastic particles from the gut of *N. norvegicus* per prawn ground ($N = 262$).

Table 3
Comparison of characteristic bands between sample and reference polymers (cm^{-1}). Details for characteristic bands, assignments, and vibration types were adapted from Shashoua (2008).

Sample	Compound (Common name)	Characteristic band (cm^{-1}) Sample reference		Assignment	Type of vibration[a]
A	Polyvinyl chloride	2916.90	2910	CH_2 & CH	N
		1412.00	1426	CH_2	D
		1018.92	958	C—C	N
		871.86	876	CH_2	F
B	Polyamide	3280.09	3300	N–H	N
		2916.62	2922	CH_2	A
		2849.59	2851	CH_2	S
		1557.68	1602	C═O	N
		1463.32	1541	NH/CN	D/N
		1248.061	1275	NH/CN	D/N
		024.98	1200	CH_2	EG
		717.90	700	NH & C═O	C
C	Polyethylene	2915.42	3000–2840	CH_2	AS
		2849.06	2849	CH_2	AS
		1469.97	1469	CH_2	D
		718.06	718	CH_2	F

[a] N–stretching vibration; C–out-of-plane deformation; D–in-plane deformation; E–wagging vibration; F–rocking vibration; G–twisting vibration; A–asymmetric stretching; S–symmetric stretching.

sold for human consumption.

All treatments, except for HNO_3, at all temperatures were within the optimum digestion rate, which allow the presence negligible amount of residue and other undigested matter (Karami et al., 2017a, 2017b), including the cuticular lining of the stomach, as part of the highly specialized digestive-tract of sampled species *N. norvegicus* (Welden et al., 2015). Even though KOH + T20 treatment is proficient at digesting biological material, it is not recommended due to the observed flocculation, causing to the development of more residue in the filter membranes that hinders both the filtration and visualization processes.

Recovery rates were tested using six synthetic polymers stained with Nile-red solution, which cover the most commonly manufactured and used plastics types (PlasticsEurope, 2018) and represent the majority of plastic particles likely to be found in environmental conditions. In accordance to the findings of Maes et al. (2017), the NR staining produced distinctively different colour ranges (light to dark red) for each type of polymers, which were all initially transparent and whitish. As observed, the solvent (acetone) partially melted the Polystyrene (PS) polymers. Tamminga et al. (2017) reported that the subsequent melting of the polymer could be attributed to the specific influence of the solvent depending on the surface exposure intensity and density properties of the synthetic material. Nevertheless, it is confirmed that the use of NR staining method is less time-consuming and effective for conducting recoverability analysis of spiked polymers, considering the type of environmental samples being analysed.

Consistent with the results of Karami et al. (2017a, 2017b) and

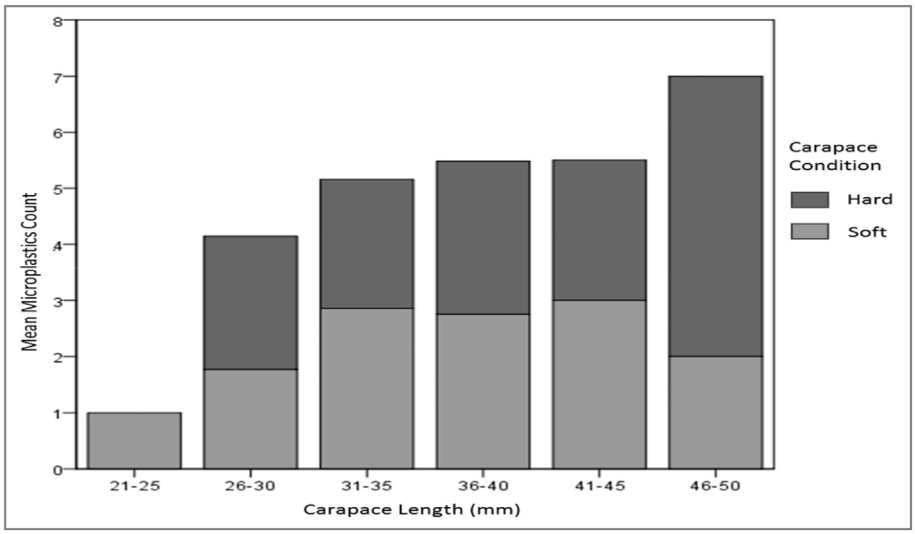

Fig. 9. Mean frequency distribution of ingested microplastics in relation to carapace length.

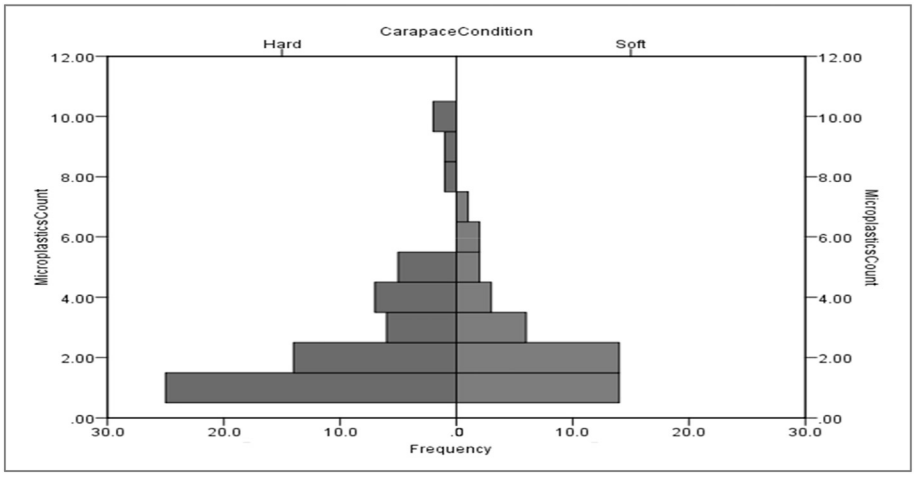

Fig. 10. Frequency distribution of ingested microplastics depending on carapace condition (hard, soft,), taken as a simple measure of the moulting stage of each individual.

Table 4
Estimated exposure to microplastics from the consumption of *N. norvegicus* in Ireland. Different scenarios are provided based on the efficiency of removal of the gastrointestinal tract.

Microplastic concentration (Particles/individual)		Estimated exposure (particles/year) As per consumption/concentration scenarios[a]		
		Once a month	Once a week	Once a day
Gastrointestinal tract (GT) intact	1.75	147	637	4471
50% removed	0.875	74	319	2236
90% removed	0.175	15	64	447

[a] Assumption: 1 tailed prawn is approximately 7 g, based on EC marketing size grade III (121–180 count per kilogram of tailed prawns) and number of tested samples (n = 150).

Dehaut et al. (2016), the digestion protocols using concentrated HNO_3 resulted in the complete degradation of Polyamide (Nylon 6,6), with significant changes in the physical structure and changes in colour (yellowing) of all other spiked polymers. Claessens et al. (2013) also reported the destructive effect of the concentrated solution, as well as its capability to fully dissolve PA 6,6, which mainly represents a commonly produced (Lithner et al., 2011) and predominantly ingested polymer types (Lusher et al., 2013). The degrading effect of the oxidizing agent could be due to its influence on the monomer composition (Lithner et al., 2011) and level of tolerance to an acidic solution of the polymers (Cole et al., 2014). As observed by Van Cauwenberghe and Janssen (2014), results of spectroscopic analysis of microplastics extracted in tissue samples using concentrated HNO_3 showed low spectral quality characterized by the disappearance of characteristics of peaks/bands of the polymer, indicating its unsuitability and inefficiency as a digesting solution (Claessens et al., 2013). Therefore, the use of HNO_3 for the digestion of biological materials such as digestive contents is not recommended.

4.2. Microplastic ingestion rates and polymer types

The proportion of *N. norvegicus* to have ingested microplastics (69%) was similar to the ingestion rate recorded by Welden and Cowie (2016a, 2016b), working on wild populations sampled in North and West Scotland, where 67% of samples had ingested microplastics. Murray and Cowie (2011) who studied 120 individuals from the Clyde Sea reported a comparably higher prevalence record of microplastics in the gut (83%). Similarly, Devriese et al. (2015) noted the presence of synthetic particles in 63% on another decapod crustacean species (*Crangon crangon*) sampled from the southern North Sea and Channel Area, wherein no spatial differences were observed. Even though there were also no significant spatial differences on microplastic contamination between ground sites as recorded in this study, individuals sampled from the North Irish Sea showed higher frequency of microplastics within the gut, which is believed to be due to a combination of environmental and anthropogenic factors. As concluded by Foekema et al. (2013), a higher frequency of samples with ingested plastics signifies higher load of plastic pollution at a local level.

The lowest ingestion rate was recorded in samples from the APG, with an estimated mean burrow density of 0.29 burrows/m^2 and produced landings of approximately €96 million in 2015, thus, emphasizing its valuable contribution to the *Nephrops* fishery in Ireland (Doyle et al., 2016). Martin et al. (2017) confirmed the presence of microplastics on the upper layer of sediments within the known habitat of *N. norvegicus* along the western Irish continental shelf, and a standardized volume of samples with a mean of 7.67 MPs/station from the APG. Since microplastics have been recorded from sediment within the prawn grounds (Martin et al., 2017) there is potentially a higher risk of accumulating microplastics through food and burrowing activities or deposit- and detritus- feeding organisms such as *N. norvegicus* (Murray and Cowie, 2011; Wright et al., 2013).

The type of ingested microplastic by *N. norvegicus* recorded in this study mainly composed of fibres is clearly similar to the most prevalent type of microplastics accumulated in sediments within the same study area (Martin et al., 2017). Other studies conducted related to the microplastic uptake of decapod crustaceans also reported that microfibers with varying size ranges and colours were predominantly detected (Murray and Cowie, 2011; Welden and Cowie, 2016a, 2016b). In comparison with other species with same functional groups and feeding preferences, size classification of ingested microplastics commonly within 1–2 mm was bigger than the range recorded for *C. crangon* (0.2–1.0 mm) (Devriese et al., 2015). As with other commercial species like mussels, cultured *M. edulis* was recorded to have a smaller MPs size range predominantly within the 5 to 10 μm (Van Cauwenberghe and Janssen, 2014).

Similarly, both Welden and Cowie (2016a, 2016b) and Murray and Cowie (2011) reported that the predominantly detected category was composed of entangled microplastic fibres. It is known that different extraction techniques being applied could also lead to dissimilarities in the abundance records due to its different detection limits even using the same type of tested samples (Li et al., 2016). Low extraction yield and potential underestimation could happen when a direct visual

sorting approach was used, as the presence of organic matter could interfere in the visualization process (Avio et al., 2015). However, it is less time consuming and relatively cheap, but requires training and expertise to not compromise the detection accuracy, as the approach is only known to be most effective for particles > 500 μm (Lusher et al., 2017a, 2017b). Therefore, it is highlighted another potential advantage of the digestion protocol employed in the present study as compared to the direct visual examination of the gut samples.

Evidence has revealed that ingested microplastics may be retained in the digestive tract, embedded in tissues or egested through different mechanisms (Browne et al., 2007). Egestion process could prevent any unfavourable effects caused by the ingestion of plastic particles to the organism (Wright et al., 2013). In the case of *N. norvegicus*, the ingested microplastic particles can be excreted through ecdysis, and the process of moulting is assumed to be the key route of excreting microplastics in prawns (Welden and Cowie, 2016a, 2016b). However, frequency of moulting varies between sexes (Bell et al., 2006). After the estimated size at onset of maturity, male *N. norvegicus* with carapace lengths between 29 and 46 mm is assumed to have a moulting frequency of 1–2 moults/year. While, females with carapace lengths around 21–34 mm only undergo 0–1 moults/year (Tuck et al., 2000; Bell et al., 2006). In contrast to the findings of Welden and Cowie (2016a, 2016b), the current results revealed that larger animals tested had high loads of microplastics in their gut in spite of its low correlation coefficient values which are within +0.3. For instance, the greater prevalence of microplastics (6–10 particles/individuals) were all found in samples with carapace length larger than 30 mm, and hard carapace condition which is assumed to be at the intermoult stage. However, no significant association between moulting stages and ingestion rate was recorded between the tested samples. As reported by Welden and Cowie (2016a, 2016b), stages of moulting have a significant effect on the aggregations of plastic particles, wherein recently moulted samples contained significantly lower levels of microplastics. These results, however, cannot be fully verified in this study since there was no jelly or recently moulted individuals found in the sample, only soft and hard carapace individuals were analysed. Lusher et al. (2017a, 2017b) mentioned that while assessing wild population, the plastic contamination can't be attributed directly to either biological responses or condition due to the manifestation of various confounding factors.

FTIR results revealed that *N. norvegicus* have ingested a variety of polymer types. Majority of the tested subset of ingested fibres from all sites were mainly identified as Polystyrene; however, NIS had the highest number of polymers identified within a single station. The other extracted polymer types, particularly the PA, PP and PVC were also reported as the most frequently observed polymers in a similar study conducted by Welden and Cowie (2016a, 2016b), while some were also found in waters and sediments within the assessed prawn ground sites (Martin et al., 2017). These records are not so surprising since it is considered as among the most demanded polymer types based on the recent analysis on the European plastics production, demand and waste data (PlasticsEurope, 2018). Ingested synthetic fibres have been analytically linked to local fishing gear types (Martin et al., 2017), as it is indicated that main fishing equipment such as nets and floats are made from a range of polymers, including PP, PE, PVC, PS and PA (UNEP, 2016). According to Browne et al. (2007), plastic fragmentation within the marine environment could be a consequence of photolytic, mechanical, and biological degradation, as well as combined effects of physical forces such as wave action and abrasion from sediment particles. However, the environmental fate of these fragmented microplastics depends primarily on its polymer density (Lusher et al., 2017a, 2017b).

The spectral characteristic bands and peaks of the representative of extracted MPs slightly differ in comparison to the spectra of pure materials presented by Shashoua (2008), probably due to the degradation process. There were also similar trends observed in the spectra, possibly an effect of the use of the digesting solution (KOH). According to Haghnazari et al. (2014), O−H stretching vibrations produces intensive bands at 3095 and 3301 cm^{-1}. Kühn et al. (2017) confirmed that no degradation occurred to different plastic types using KOH treatment and is considered as a suitable approach for quantitative studies of plastic ingestion in marine organisms. Dehaut et al. (2016) tested the same digesting protocol for tissues of mussels, crabs and fish, showing no evident impact on polymer mass or form.

In terms of contamination control, the level of particles in the procedural blanks ensured the reliability of the strict measures employed to eliminate any possible contamination, which was below the limit of detection (LOD) values set for airborne fibres as specified by De Witte et al. (2014). The airborne particles recorded throughout the laboratory analyses were relatively smaller as compared to the most common size class of microplastics extracted from the samples.

4.3. Potential microplastics exposure and risk from the consumption of seafood

The consumption of seafood products, particularly those that are being consumed with intact gastrointestinal tract, represents an exposure pathway of microplastics to humans (Smith et al. 2018). In order to calculate the dietary exposure to microplastics and communicate the associated risks that have implications for food safety, relevant information on consumption and ingestion are needed, taking into consideration the estimated intake of the seafood product as well as the concentration of microplastics (Lusher et al., 2017a, 2017b). For instance, Van Cauwenberghe and Janssen (2014) estimated that the top consumers of cultured bivalves in Europe have a dietary exposure amounting to 11,000 MPs/year. Other research includes exposure to microplastics in four commonly consumed dried fish from local markets in Malaysia, wherein consumers are expected to ingest 6–246 MPs/year considering the average weight and the number of plastic particles per individual fish (Karami et al., 2017a, 2017b).

The consumption of seafood such as crustaceans and mollusks were variable among countries in Europe (EFSA, 2014). In general, consumption of fisheries and aquaculture product varies from 4.8 kg/person in Hungary to 55.9 kg/person in Portugal, while 22.1 kg/person annually was recorded in Ireland (EuroStat and Eumopa, 2015). Based on the report about the snapshot of Ireland's seafood sector, the consumption of seafood is relatively low compared with other European countries. However, Irish consumers were known to purchase prawns most frequently (BIM, 2015), accounting for 84% of the total consumption of crustaceans, with an estimated dietary intake of 7.0 g/day for adult consumers, aging from 18 to 64 years old (EFSA, 2014).

The exposure assessment of microplastics in prawns confirmed that the estimated intake of microplastics per adult consumer annually is approximately within 15–4471 particles. These values were selected in order to portray the worst to best case scenarios of consuming prawns. Similar best-case scenario with Devriese et al. (2015) was used to determine the exposure considering 90% removal of ingested microplastics by peeling since it is known that the stomach of *N. norvegicus* are usually being discarded and not eaten directly (Murray and Cowie, 2011). As expected, this assumption indicated a relatively low potential exposure to microplastics (15 to 447 MPs/year). However, exposure analysis using the highest and lowest averages of microplastic concentration across the assessed ground sites, the KB (2.30 MPs/ind) has an estimated range of 19–588 MPs/year, while APG (0.9 MPs/ind) will result in an estimated exposure between 8 and 230 MPs/year. These indicative results are still comparably higher when compared to the exposure assessment made for another decapod crustacean species, brown shrimp *C. crangon*, in which average consumers were only expected to ingest between 15 and 175 MPs/year (Devriese et al., 2015). Consequently, a maximum number of about 450 particles per year appear to me more realistic.

The results of this baseline study confirmed the prevalence and abundance of microplastics in the wild populations of *N. norvegicus* in

Ireland, as similar to what was previously shown by Murray and Cowie (2011) for Scottish waters. However, it is recommended that future studies on the same species should adopt the methodologies outlined here, which are more robust, cheaper when compared to enzymatic digestion, and allow for comparison between studies, particularly those focused on long-term monitoring.

5. Conclusion

Strategic monitoring and quality assessment of a selected key species may improve our understanding of the mechanisms, patterns and hotspots of microplastics contamination on a local level. The prevalence of microplastic ingestion in *N. norvegicus* indicates that future research across a wide range of both commercial and non-commercial species and their habitats should be considered to fully establish the current baselines and the potential consequences of microplastics in the marine environment, taking into account a higher number of individuals, broader spatial coverage and/or temporal aspects. As seafood, such as prawns, is consumed by humans worldwide, the presence of microplastics in wild stocks potentially poses a risk to food safety. Therefore, the estimates of dietary exposure to microplastics as prescribed in this study, could provide relevant information and evidence to concerned authorities as well as consumers, relevant to effective decision making and appropriate measures in controlling any possible risks linked to seafood safety.

CRediT authorship contribution statement

Jenevieve Hara: Conceptualization, Project administration, Methodology, Investigation, Data curation, Writing - original draft. **João Frias:** Conceptualization, Methodology, Supervision, Writing - review & editing. **Róisín Nash:** Conceptualization, Methodology, Supervision, Writing - review & editing.

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.

Acknowledgements

The authors would like to acknowledge the International Master of Science in Marine Biological Resources (IMBRSea), funded through the Erasmus Mundus (Partner Country) Scholarship (reference 20170403) of the European Commission. This research project was supported by the Irish Marine Institute in the collection of samples for this research and the IMP.act Project (Managing for Microplastics: A Baseline to Inform Policy Stakeholders) funded by the Irish Research Council (IRC) under the framework of the Marie Skłodowska-Curie Actions (MSCA) COFUND Collaborative Research Fellowships for a Responsive and Innovative Europe (CAROLINE) scheme (fellowship reference CLNE/2018/524).

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