Marine Pollution Bulletin 180 (2022) 113768

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

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

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ARTICLE INFO

Keywords:
Microplastic
Egestion
Pollution
Nephrops norvegicus
Retention

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 h 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*.

1. 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 12 h (Hoang and Felix-Kim, 2020), while the Brine Shrimps, *Artemia*, ranged between 2 and 72 h (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

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https://doi.org/10.1016/j.marpolbul.2022.113768
Received 21 December 2021; Received in revised form 19 April 2022; Accepted 15 May 2022
Available online 24 May 2022

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 8100 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.

2. Materials and methods

2.1. 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.

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 acclimatization 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.

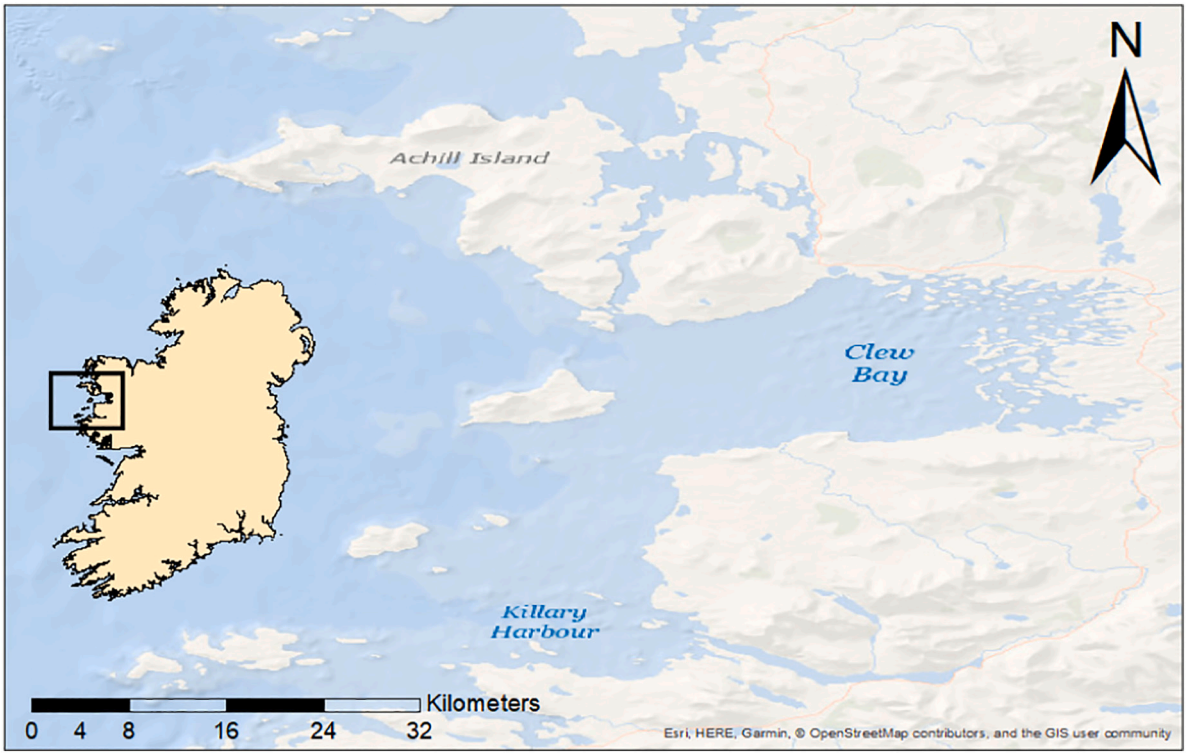

Fig. 1. *N. norvegicus* sampling site on Clew Bay, Bellmullet, Co. Mayo.

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 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. 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 h. 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.

2.2. 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 h, and then again at 24 h. 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.

2.3. Fibres and feed

Outside of the baseline the organisms (n = 46) were divided into three experimental groups (Fig. 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 (75 mg of Nile Red stock solution with 75 mL 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 min 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.

2.4. Microplastic exposure trial

The retention experiment took place over three separate trials (Fig. 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 24 h 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 47 mm Whatman® (GF/C – 1.2 μm pore size) glass microfiber filter paper after the experiment.

		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

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

2.5. 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 h, as recommended by Hara et al. (2020). The digestive solution was filtered using a vacuum pump (VCP130) through 47 mm 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.

2.6. 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. 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 56 mm, with a mean of 47.76 ± 3.92 mm. The organisms had a mean total length (TL) of 120.2 ± 18.7 mm and a mean mass of 49.8 ± 14.4 g. 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.

3.1. Egestion rates and behaviour

The baseline analysis of food egestion saw all organisms that consumed food, egest the food between 6 and 24 h. Subsequently, the first sampling time in the MP feeding experiment was based on this 24 h. 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.

3.2. 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) (Fig. 3). The fibres not accounted for in the stomachs of *N. norvegicus* were retrieved from the filtered water in the experimental tanks.

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 (Fig. 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.

4. 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 h after food ingestion (Sardà and Valladares, 1990). To ensure sufficient time for organisms to egest seeded food, a sampling interval of 24 h was chosen. A previous short-term feeding experiment of 24 h, 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

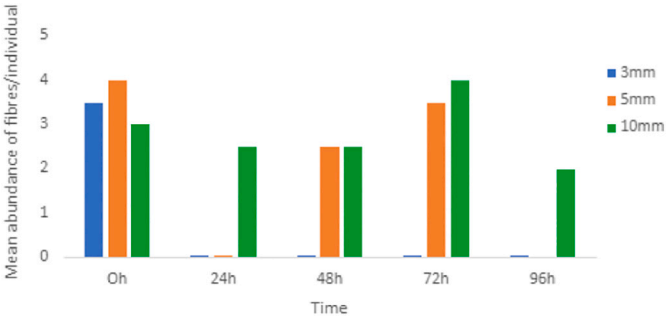

Fig. 3. Mean MP retention in *N. norvegicus* of polyester fibres of three sizes (3, 5, and 10 mm).

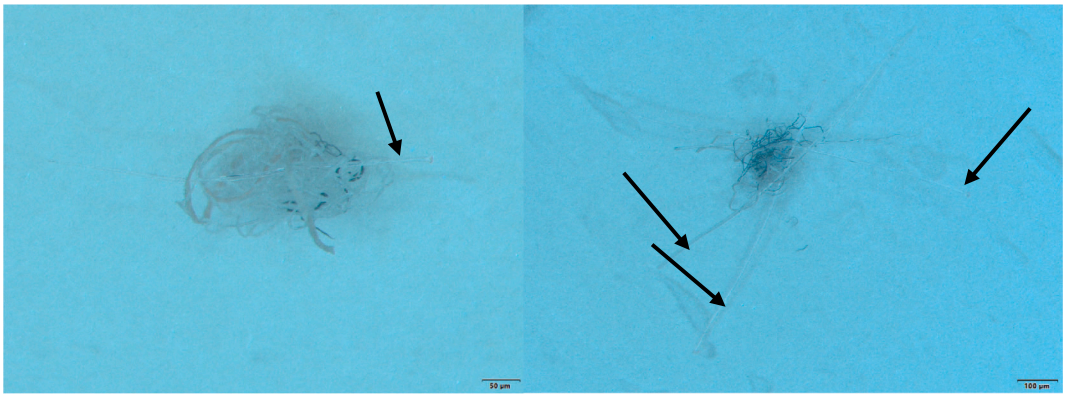

Fig. 4. Two entangled balls of fibres extracted from *Nephrops norvegicus*, where an introduced MP from feeding (highlighted with an arrow). Left: 5 mm fibre recovered at t72 (trial 2) from the foregut of a female. Right: 10 mm fibres ($n = 3$) recovered at t72 (trial 3) from the foregut of a male.

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 h after ingestion for 10 mm fibres and 72 h 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 h 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.

5. 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. **Fiona Kavanagh:** Writing – review & editing, Supervision. **Thomas Power:** Investigation. **Jonathan White:** Writing – review & editing. **João Frias:** Conceptualization, Methodology, 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.

Acknowledgements

The authors would like to acknowledge the Marine Institutes and the European Maritime and Fisheries Fund (EMFF) Marine Biodiversity Scheme. The “Nephrops and Microplastics” project (MB/2018/04) is part of the Marine Biodiversity Scheme which is carried out under Ireland's Operational Programme (OP), co-funded by the European Maritime and Fisheries Fund (EMFF) and by the Irish Government.

The authors would also like to acknowledge Dr. Anita Talbot and Ms. Laura Maria Vilchez-Padial from the MFRC for their technical support while setting up the tanks in the laboratory for this experiment.

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