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Microplastic interactions with North Atlantic mesopelagic fish

Amy L. Lusher[1]*, Ciaran O'Donnell[2], Rick Officer[1], and Ian O'Connor[1]

[1]*Marine and Freshwater Research Centre, Galway-Mayo Institute of Technology, Dublin Road, Galway, Ireland*
[2]*Marine Institute, Rinville, Oranmore, Co. Galway, Ireland*

**Corresponding author: tel: + 353 91 742501; fax: +353 91 751107; e-mail: amy.lusher@research.gmit.ie*

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Microplastics in the marine environment are well documented, and interactions with marine biota have been described worldwide. However, interactions with vertically migrating fish are poorly understood. The diel vertical migration of mesopelagic fish represents one, if not the largest, vertical migration of biomass on the planet, and is thus an important link between the euphotic zone, transporting carbon and other nutrients to global deep sea communities. Knowledge of how mesopelagic fish interact and distribute plastic as a marine contaminant is required as these populations have been identified as a potential global industrial fishery for fishmeal production. Ingestion of microplastic by mesopelagic fish in the Northeast Atlantic was studied. Approximately 11% of the 761 fish examined had microplastics present in their digestive tracts. No clear difference in ingestion frequency was identified between species, location, migration behaviour, or time of capture. While ingesting microplastic may not negatively impact individual mesopelagic fish, the movement of mesopelagic fish from the euphotic zone to deeper waters could mediate transfer of microplastics to otherwise unexposed species and regions of the world's oceans.

Keywords: ingestion, myctophids, plastic pollution, trophic effect, vertical transport.

Introduction

Marine debris, the majority of which is plastic, has been documented worldwide and can have negative effects and impacts on marine biota (Derraik, 2002). Large plastic items can cause entanglement and be ingested by organisms with visible implications. Smaller plastics can affect organisms through respiration, ingestion, gastric obstruction, physiological effects, chemical transfer, or trophic transfer (Lusher, 2015). Microplastics [<5 mm; defined by Arthur *et al.* (2009)] are ingested by commercially important invertebrate species, including Norway lobsters (*Nephrops norvegicus*, L. 1758; Murray and Cowie, 2011) and bivalves (e.g. *Mytills edulis*; De Witte *et al.*, 2014). Microplastic ingestion by fish is not an uncommon observation (Lusher, 2015). For example, microplastics were found in the stomachs of commercially caught fish and mesopelagic fish, from the English Channel and the North Pacific (Boerger *et al.*, 2010; Lusher *et al.*, 2013). Recently, microplastics were isolated from copepods and Euphausiids (Desforges *et al.*, 2015), which, if ingested by predatory species, could facilitate trophic transfer to organisms higher in the food chain. To understand the implications of microplastics (distribution in the marine environment and subsequent interactions with biota), as ubiquitous contaminants in a global context, it must be emphasized to study organisms which are likely to be exposed to microplastics: those whose distributions overlap in time and space with the distribution of microplastic. When such organisms are also of commercial interest, either as direct sources of protein or derivatives (e.g.: fish oils), they could increase human exposure to microplastics is an additional concern.

Plastic levels in the Northeast Atlantic are relatively unknown; however, a recent study found that microplastics were ubiquitous in subsurface waters with an average of 2.46 microplastics per m^{-3} (Lusher *et al.*, 2014). As particles can accumulate within highly productive ocean features such as gyres [for review, see Lusher (2015)], microplastics may mix within the neuston and planktonic food sources of pelagic species.

There are nearly 1100 species of fish in the North Atlantic, of which 600 are pelagic (Merrett, 1995). Extensive pelagic fisheries occur in the Northeast Atlantic for Atlantic mackerel (*Scomber scombrus*, L. 1758) and blue whiting (*Micromesitius poutassou*, L. 1758), which are known to undertake large-scale spawning migrations (Bailey, 1982; Reid *et al.*, 2006). Taken together, annual catches account for >2 million tonnes (Marine Institute, 2014). Mesopelagic fish are known to form part of the diet of mackerel and blue whiting linking the wider open Atlantic with shelf seas (Olaso *et al.*, 2005; O'Donnell *et al.*, 2013).

Recently, it has been suggested that the large quantity of mesopelagic fish in the Atlantic are of commercial value, and could be targeted in response to the growing demand from aquaculture for fish proteins and oil (FAO, 2010). Mesopelagic fish are an important component of the oceanic mesopelagic ecosystem in the North Atlantic (Gjøsæter and Kawaguchi, 1980). Mesopelagic fish have global biomass estimated at between 600 and >1000 million metric tonnes (Gjøsaeter and Kawaguchi, 1980; Irigoien *et al.*, 2014) and are one of the last marine fish resources that are still underutilized by fisheries. Mesopelagic fish are important predators of zooplankton (e.g. Gjøsæter, 1973) and prey for fish (e.g. Olaso *et al.*, 2005), seabirds (e.g. Danielsen *et al.*, 2010), and marine mammals (Spitz *et al.*, 2006; Pusineri *et al.*, 2007; Hernández-Milián, 2014). Mesopelagic fish play an important role in oceanic energy dynamics, linking primary consumers to higher predators, birds, fish, and marine mammals. Mesopelagic fish also act as a pathway for carbon transport, exporting carbon from the surface to the deep ocean through migration patterns and faeces production (Davison *et al.*, 2013).

Mesopelagic fish reside at ocean depths, primarily below the photic zone during daylight hours, and carry out diel vertical migrations (DVMs) to exploit zooplankton near the surface at dusk and dawn, when light intensities are sufficient for visual predation on plankton but sufficiently low to protect against predation (Clark and Levy, 1988). As microplastics appear to aggregate in surface waters, mixing with neuston, there is a likelihood that fish feeding in surface waters may be unable to distinguish between plastic particles and their target food sources. For example, mesopelagic fish ascending to surface waters following zooplankton diurnal migrations are exposed to higher levels of microplastics due to the accumulation of plastics in surface waters. Microplastics could be mistaken for prey as they have similar shapes and forms as prey items, and are ubiquitous within the floating planktonic community [for review, see Lusher (2015)].

Ingested microplastics could artificially be transported within the water column to depths that would not normally be achievable due to their physical characteristics (for example, buoyancy). Furthermore, if microplastics are retained within fish, they could act as a source of microplastics to larger marine organisms, including predatory fish and marine mammals feeding below the photic zone or near surface waters during nightfall (Figure 1). For example, in the Northeast Atlantic, mesopelagic make up 39–65% of the diet of striped dolphins (*Stenella coeruleoalba*, M. 1833; Spitz *et al.*, 2006; Hernández-Milián, 2014), and spotted lanternfish (*Myctophum punctatum*, R. 1810) and lancet fish (*Notoscopelus kroyeri*, M. 1861) are the main prey species of the common dolphin (*Delphinus delphis*, L. 1758) (Pusineri *et al.*, 2007; Brophy *et al.*, 2009). Beaked whales including True's beaked whales (*Mesoplodon mirus*, T. 1913) show a preference for mesopelagic fish (Pauly *et al.*, 1998). While microplastics were found in the digestive tract of a True's beaked whale, it is uncertain whether these microplastics originated from prey items (Lusher *et al.*, 2015). Microplastics found in the scats of fur seals (*Arctocephalus* spp., F.V. 1826) are believed to have originated from rough lanternfish (*Electrona*

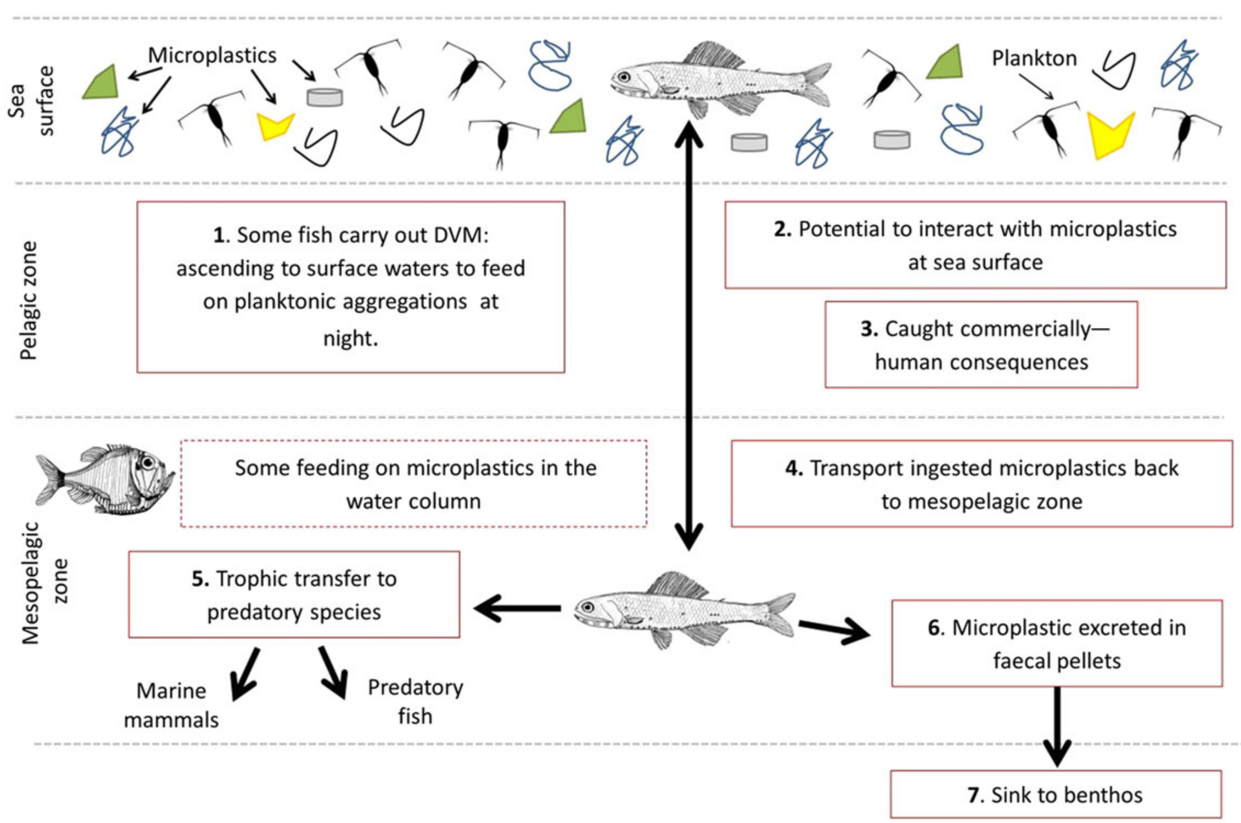

Figure 1. Conceptual model of mesopelagic fish interactions with microplastics and subsequent transferal to depths. DVM, diurnal vertical migration.

subaspera, G. 1864; Eriksson and Burton, 2003). However, before such links are confirmed, the exposure threat of mesopelagic fish to microplastics requires examination. Furthermore, species such as *N. kroyeri* and *M. punctatum* are potential target species for fisheries as they are highly abundant throughout the Atlantic. An initial assessment of their potential plastic load is required to understand the potential for microplastic transfer to secondary consumers.

The study sought to determine the occurrence and types of microplastics ingested by mesopelagic fish from open waters of the Northeast Atlantic. The primary aim was to understand the levels of microplastic ingestion, with a secondary aim being to determine whether there was any difference in the frequency of ingestion between species and locations. After consideration of the feeding habits of many species, it was hypothesized that fish caught at night would have more microplastics in their digestive tracts than those caught during the day. Additionally, it was suggested that fish caught during the day containing microplastics could show retention in their digestive tracts. Finally, the potential consequences of microplastic ingestion by mesopelagic fish on marine foodwebs are discussed.

Methods

Sample collection

Fish sample collection was carried out on the RV *Celtic Explorer* during the blue whiting acoustic survey (BWAS) in 2013 and 2014. Mesopelagic samples composed of: (i) opportunistic bycatch when targeting blue whiting aggregations ($n = 11$) and (ii) dedicated directed trawls on mesopelagic layers that were identified in shallower depths ($n = 4$). Haul positions and details can be found in Table 1. Trawl sampling was carried out using a single pelagic midwater trawl with a vertical opening of 55 m and a circumference of 768 m fitted with a 20-mm codend liner. For all surveys, fish were sorted to species level and up to 50 individuals of each species were collected per haul. Fish otoliths were used to confirm species identity. Incorrect identification of species on initial count led to two hauls with >50 individuals per species analysed. The 10 species of mesopelagic fish used in this analysis include *Benthosema glaciale* (R. 1837), *M. punctatum*, *N. kroyeri*, *Lampanyctus crocodilus* (R. 1810), *Maurolicus muelleri* (G.1789), *Stomias boa boa* (R. 1810), *Nemichthys scolopaceus* (R. 1848), *Arctozenus risso* (B. 1840), *Xenodermichthys copei* (G. 1884), and *Argyropelecus* spp. As catch rates varied between successive trawls (Supplementary Information 1), the numbers of individuals per species available for analysis were not standardized. Microplastics from water samples were collected during the BWAS 2013 and 2014 [for method, see Lusher *et al.* (2014)].

Sample preparation

Fish were identified and frozen within 1 h of capture, and subsequently thawed at room temperature before examination in the laboratory. For each fish, basic measurements included length, from mouth to central point of caudal fin (mm) and body weight (g). Digestive tracts were removed by dissection from each fish, from the top of the oesophagus, and cut away at the vent following Lusher *et al.* (2013). Digestive tracts were dissolved in 10% KOH following Foekema *et al.* (2013) on removal from the fish. After being in the solution for 2 weeks, the remaining material was filtered through a 250-μm mesh.

Microplastic identification

Particles retained on the mesh were visually inspected under a stereomicroscope with a polarizer attached (Olympus SZX10 with a mounted Q-imaging Retiga2000R camera, ×2.5 magnification). Photographs of all potential microplastics were recorded along with maximum length (mm), shape, and colour. Visual classification of particles was carried out using established criteria (Lusher *et al.*, 2014). For particles to be classified as plastic, they had to (i) be homogenously coloured, (ii) be shiny and not matt, (iii) have no cellular or organic structures visible, (iv) be equally thick throughout their length, and (iv) fibres had to have three-dimensional bending. Where possible, a saturated salt solution was used to check for positive density of larger items. Any particles suspected of being of a cellulosic or semi-synthetic form were rejected from further analysis. Particles were assigned to two particle type categories: fibres and fragments.

Contamination prevention

Work surfaces were thoroughly cleaned with alcohol, and hands and forearms were scrubbed. Lab coats, cotton clothing, and gloves were worn when working to reduce contamination. All manipulation instruments and equipment were cleaned and checked under a microscope for contamination with airborne fibres before use. To minimize the risk of contamination, fish were opened with a scalpel and digestive tracts were immediately placed in clean collecting

Table 1. Haul details from mesopelagic fish collection in the Northeast Atlantic during the BWAS.

Haul	Date	Duration (h:min)	Latitude (° North)	Longitude (° West)	Target depth range (m)	Day or night
BWAS 1	7 April 2013	00:45	60.600	3.585	490–520	Day
BWAS 2	8 April 2013	00:46	61.366	3.716	500–520	Day
BWAS 3	8 April 2013	00:35	60.718	3.408	80–120	Night
BWAS 4	9 April 2013	00:38	59.242	7.255	260–280	Day
BWAS 5	10 April 2013	01:22	58.798	7.989	60–100	Night
BWAS 6	11 April 2013	00:54	54.808	10.589	130–200	Day
BWAS 7	11 April 2013	00:35	54.270	11.576	20–100	Night
BWAS 8	27 March 2014	00:30	53.420	14.517	537	Day
BWAS 9	28 March 2014	00:32	54.296	13.046	500	Night
BWAS 10	30 March 2014	00:12	55.306	12.834	500	Day
BWAS 11	30 March 2014	00:17	55.805	9.587	477	Night
BWAS 12	31 March 2014	00:12	55.803	10.962	450	Day
BWAS 13	4 April 2014	01:16	58.803	8.102	420	Day
BWAS 14	4 April 2014	00:30	58.799	10.725	500	Night
BWAS 15	5 April 2014	00:43	59.301	7.149	250	Night

vials. Controls were conducted randomly throughout the sample analysis. To check for airborne contamination, clean, contamination-free Petri dishes, and filter papers were left exposed to air during sampling in the laboratory. Replicate control filter papers, held under a Petri dish, were left exposed to air during the laboratory work. Contamination during the vacuum filtering stage was assessed by passing pre-filtered water through a clean GF/C filter paper. Contamination during dissolution was tested using pre-filtered water. As a small number ($n = 5$) of matt black fibres were found on filter papers ($n = 30$), particles with similar size, colour, and structure were rejected from subsequent analysis. As the contamination controls did not have coloured fibres, coloured fibres were not discounted. Airborne contamination was considered to be negligible.

Statistical analysis

The proportion of fish with microplastic in their digestive tract was calculated for: (i) all species individually and (ii) all fish in the whole sample due to the large variability in the number of individuals per species. The average number of microplastics ingested was calculated for fish that ingested plastic (11% of data). The frequency of ingestion for species caught at different times of day was compared with the non-parametric Kruskal–Wallis test (as data were not normally distributed). Count data for the number of plastics ingested were heavily weighted by single values for plastics and transformations did not normalize the data. As such, non-parametric analyses were conducted. The proportions of species ingesting plastic at night and day were compared using Kruskal–Wallis to test for differences between times of the day. To compare plastic levels in fish to the surrounding environment, the average number of microplastics per fish from each haul caught in the upper water column (<100 m) was compared with the average microplastic per m^3 in the surrounding subsurface seawater collected as part of Lusher *et al.* (2014). As this data subset was normally distributed (Kolmogorov–Smirnov: $p < 0.05$) and exhibited homogenous variance, a Pearson's correlation test was performed. The number of ingested microplastics determined during this study were then used as a proxy to extrapolate the microplastic load in mesopelagic fish communities on a global scale, using estimates of biomass proposed by Gjøsaeter and Kawaguchi (1980) (550–600 million tonnes).

Results

Plastic occurrence

Of the 15 trawls for mesopelagic fish, eight were conducted during the day and seven during the night (between dusk and dawn). A total of 761 individuals were caught from six families (Table 2). The most commonly caught species were *N. kroyeri* (54.8%), *M. muelleri* (37.1%), *B. glaciale* (3.6%), *A .risso* (1.9%), *Argyropelecus* spp. (0.6%), *S. boa boa* (0.6%), *X. copei* (0.6%), *M. punctatum* (0.4%), *L. crocodilus* (0.3%), and *N. scolopaceus* (0.1%). In total, 84 of 761 individuals (11.0%) contained plastic in their digestive tract. Species ($n > 10$) with highest percentage ingestion included *B. glaciale* (22%), *A. risso* (21%), and *N. kroyeri* (14.8%). Individuals from vertically and non-vertically migrating taxa had ingested microplastics, although no plastics were found in *L. crocodilus* ($n = 2$), *M. punctatum* ($n = 3$), or *Argyropelecus* spp. ($n = 5$). Of the 84 individuals that did ingest plastic, there was on average 1.2 microplastic particles per individual ($\pm$ 0.54 SD). In comparison, the average number of microplastics ingested by total fish sampled ($n = 761$) was lower (0.13 items per individual).

There was no observed difference between the percentage of fish that had plastic in their digestive tracts at night (10.3%) compared with those during the day (11.5%; Figure 2a). When disaggregated by species, the average proportion of fish ingesting plastic was higher at night (0.22) than during the day (0.14; Figure 2b), although there was no significant difference between the proportion of individuals per species with plastic at different times of day (Kruskal–Wallis, $h = 0.76$, d.f. $= 1$, $p = 0.39$). Of the most predominantly caught species, *B. glaciale* had a higher percentage of individuals ingesting plastic at night, whereas *N. kroyeri* and *M. muelleri* had more microplastic during the day and at lower percentages (Figure 3).

There was no observed relationship between the average number of microplastic ingested per individual and the average number of microplastics in the surrounding seawater as determined from water sampling (Pearson's correlation, $r^2 = 0.14$, $p = 0.86$).

Microplastic breakdown

A total of 101 microplastic particles ranging in size from 0.5 to 11.7 mm (median: 1.9 mm) were identified in the digestive tracts of mesopelagic fish. Ninety-four percentages of particles were <5 mm in length and 8% were <1 mm in length. The most prominent colours were black (42%) and blue (34%), followed by grey (10%), orange (7%), green (4%), and red (3%). Particles were identified as fibres (93%) and fragments (7%; Figure 4). In total, 101 microplastics were recovered from the digestive tract of fish samples, ranging from 0 to 4 items per individual.

Microplastic load of mesopelagic fish

It was assumed that microplastic ingestion by 11% of individuals in this study is representative of all mesopelagic fish in the North Atlantic and comparable to mesopelagic fish worldwide. Using estimates of mesopelagic biomass proposed by Gjøsaeter and Kawaguchi (1980) (550–600 million tonnes), this would equate to 60.5–66 million tonnes of fish containing microplastics. Using the average (minimum–maximum) weight of fish from this study, 5.6 g (0.1–32.0 g), microplastics could be found in 1.2×10^{13} individuals (range: 2.1×10^{12}–5.5×10^{14}) in the world's oceans at any given time.

Discussion

Levels of ingestion in the Northeast Atlantic

This study confirmed that microplastics are present in digestive tracts of Northeast Atlantic mesopelagic fish. Microplastic ingestion (11%) was similar to a study conducted in the North Pacific Subtropical Gyre (NPSG; 9.2%; Davison and Asch, 2011), an area of high open ocean plastic concentrations (Moore *et al.*, 2001). In contrast, values reported here are substantially less than in the North Pacific Central Gyre (NPCG) (35%; Boerger *et al.*, 2010). Furthermore, coastal studies of commercially targeted pelagic and demersal species showed varied levels of ingestion in the North Sea (<5.4%) and the English Channel (36.5%; Foekema *et al.*, 2013; Lusher *et al.*, 2013). Variability in ingestion may be related to the feeding habits of species as well as their proximity to urban areas. Additionally, net feeding might be responsible for the greater values of microplastic ingestion (Davison and Asch, 2011). Fine mesh sizes will retain microplastics making them available for ingestion, and longer tow durations could have a higher risk of net feeding bias than shorter tows. For example, a high percentage of ingestion was observed in the NPCG where manta net trawls

Table 2. Fish species collected during BWAS in 2013 and 2014.

Family	Species	FAO species code	Depth range (m)	Migratory	Count	Average fish length (mm) (± SD)	Percentage ingestion (%)	Average plastic per fish	Average plastic per fish with plastic
Order Anguilliformes									
Nemichthyidae	*Nemichthys scolopaceus*	ANM	200–4000	DVM[(a)]	1	870.0	100	1	1
	Order Aulopiformes								
Paralepididae	*Arctozenus risso*	NRD	200–1000	No DVM	14	190.36 ± 24.83	21	0.29	1.3
	Order Argentiniformes								
Alepocephalidae	*Xenodermichthys copei*	AXC	100–2000	DVM[(b)]	5	140.40 ± 17.49	60	1.2	2
	Order Myctophiformes								
Myctophidae	*Benthosema glaciale*	BHG	140–530 (d) 0–500 (n)	DVM	27	60.10 ± 7.61	22	0.33	1.5
Myctophidae	*Lampanyctus crocodilus*	LYD	700–1000 (d) 45–100 (n)	DVM	2	100.00 ± 45.25	0	0	0
Myctophidae	*Myctophum punctatum*	MTP	225–750 (d) 0–125 (n)	DVM	3	77.67 ± 5.01	0	0	0
Myctophidae	*Notoscopelus kroyeri*	LAX	0–1000 (d) 0–125(n)	DVM	417	99.49 ± 17.32	14.6	0.16	1.1
Order Stomiiformes									
Sternoptychidae	*Argyropelecus* spp.	SEE_1	200–800 (d) 100–600 (n)	Some DVM[(c)]	5	43.00 ± 6.78	0	0	0
Sternoptychidae	*Maurolicus muelleri*	MAV	150–200 (d) 20–40 (n)	DVM	282	53.97 ± 9.65	2.8	0.032	1.1
Stomiidae	*Stomias boa boa*	SBB	500–800 (d) >200 (n)	DVM	5	193.40 ± 37.33	40	0.8	2

FAO species codes available at http://www.fao.org/fishery/collection/asfis/en. SEE_1 code created as no FAO code available for genus level identification of *Argyropelecus* spp.; LAX genus level identification used for *Notoscopelus kroyeri* as no species level code exists. Depth range and migratory information (DVM; diel vertical migration) from www.fishbase.org.
(a) Karmovskaya 1982 in Feagans-Bartow and Sutton, 2014; (b) Roe and Badcock, 1984; (c) Roe and Badcock (1984), and references therein.

were between 1.5 and 5.5 h (Boerger *et al.*, 2010). In contrast, 15–22 min trawls were conducted in the NPSG (Davison and Asch, 2011), and 12–82 min trawls with large mesh size were deployed in this study. The likelihood of net feeding was considered negligible in this study as the sampling gear used was of commercial fishing size (55 m vertical opening), compared with the research manta net (ca. 0.5 m) or a commercial sized demersal trawl (ca. 3 m). Furthermore, the net passed only briefly and obliquely through the mesopelagic layer en route to its target depth of between 500 and 650 m (for blue whiting) in all but the dedicated mesopelagic trawls (directed specifically at mesopelagic layers/aggregations).

No correlation was found between microplastic numbers in the subsurface waters compared with the numbers of microplastics in fish caught in the same area. This suggests that fish interactions with microplastics are not necessarily as frequent as first assumed. However, reported values for microplastics in fish and the subsurface seawater may not be readily comparable. Fish are also highly mobile and could be encountering very patchy distributions of microplastics. Microplastics can accumulate at the sea surface because of the combined effects of their density, localized conditions, and larger scale oceanic processes. Thus, fish feeding in surface waters, following DVM, have a greater potential for interaction with microplastic. There is a smaller probability of interaction between fish and microplastic at depth as microplastic concentrations could be lower than at the surface.

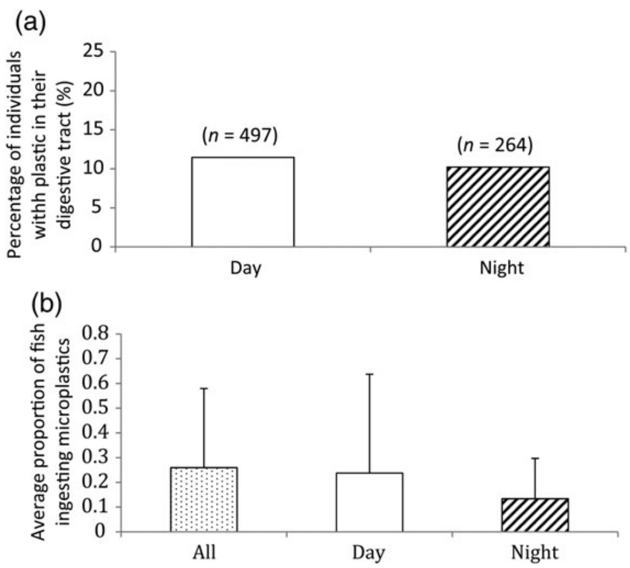

Figure 2. (a) Percentage ingestion from all species caught divided between night and day. (b) Proportion of mesopelagic fish ingesting microplastics by species, and error bars display SD of the mean.

Seasonal effects may also occur. During spring sampling, the surface waters were well mixed (O'Donnell *et al.*, 2013, 2014); however, summer sampling and the presence of a thermocline may lead to concentrated entrapment of microplastics in less-dense surface waters. Thus, interactions between mesopelagic fish and microplastics could be higher in summer than in other seasons.

Of the three species predominantly caught in this study, *B. glaciale* presented the highest percentage of microplastic ingestion. However, comparisons between these three species should be considered with caution: *B. glaciale* were caught in fewer numbers ($n = 27$) and only in a third of the hauls, whereas *N. kroyeri* were found in 87% of hauls. Although *B. glaciale* percentage ingestion was high, it is not comparable to the other species due to the large numbers of *N. kroyeri* ($n = 417$) and *M. muelleri* ($n = 282$). Most species were sampled too sporadically and in too few numbers to understand the influence of DVM on microplastic ingestion (individuals with DVM, $n = 742$ and without DVM, $n = 19$). There was also no difference between fish caught at different times of the day and the hypothesis that fish caught during night-time feeding activities are more susceptible to plastic ingestion was rejected. In contrast, Davison and Asch (2011) found that non-vertically migrating taxa had lower percentage ingestion (4.8%) compared with vertically migrating taxa (11.6%), which could indicate a decreased concentration of plastics at depths where feeding occurs. The comparison between organisms that carried out DVM and those that did not was confounded by the small numbers of

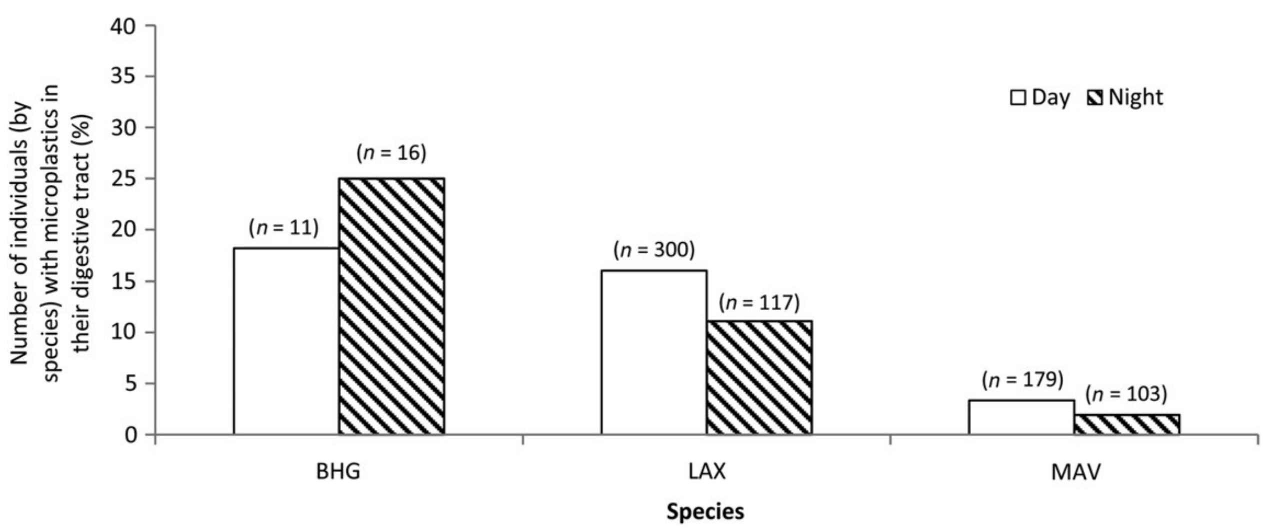

Figure 3. Percentage ingestion for the three most abundant species of mesopelagic fish from this study, divided into day and night. Species codes BHG—*Benthosema glaciale*; LAX—*Notoscopelus kroyeri*; MAV —*Maurolicus muelleri*.

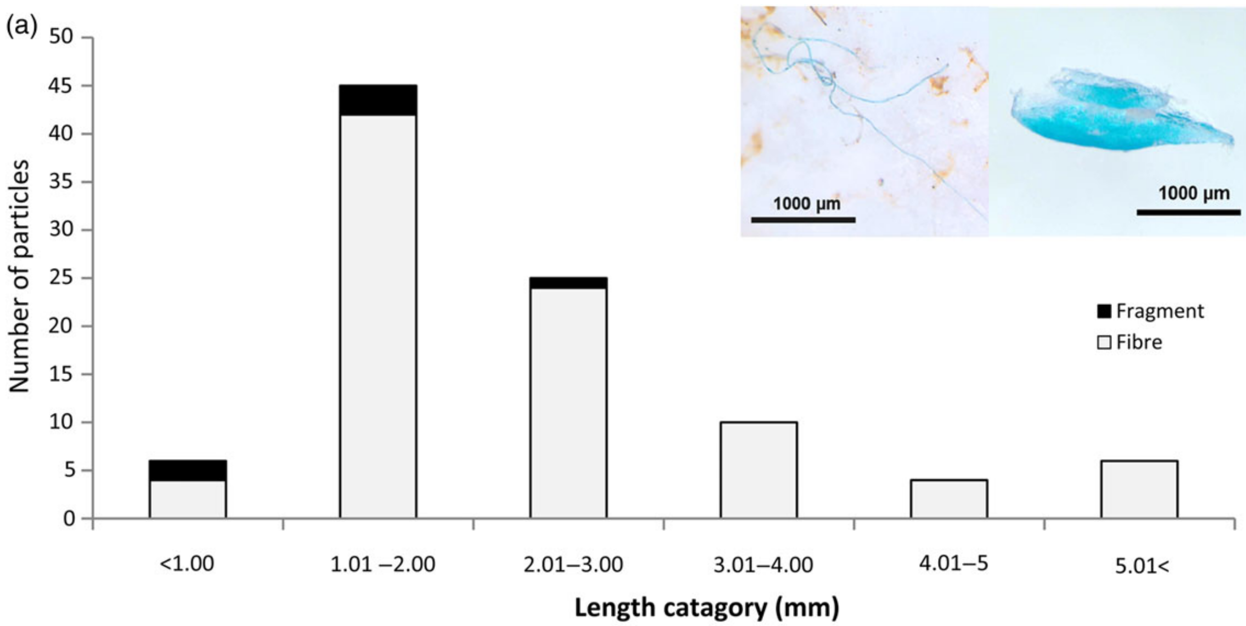

Figure 4. Breakdown of microplastics by size category. (a) Division by the type of plastic (including example images of a fibre and a fragment). (b) Division by the colour of plastics.

individuals that do not carry out DVM. Such comparisons are further complicated by populations having different migratory patterns in relation to season, size, and location. For example, populations of *Argyropelecus* spp. carry out DVM depending on populations (Roe and Badcock, 1984; and references therein) and the small number of individuals ($n = 5$) rendered it impossible to make an assessment. Additionally, *A. risso*, which do not carry out DVM, had 21% ingestion.

As there was no observed difference in microplastic occurrence between DVM taxa and non-DVM taxa, this could suggest that either:

(i) The distribution of microplastics is comparable throughout the water column, waters are well mixed, and thus mesopelagic fish are exposed to similar microplastic concentrations. Hydrographic conditions support this contention to the extent that surface waters were well mixed in both years (O'Donnell *et al.*, 2013, 2014). Also, trawl sampling did not occur directly in the surface waters (40–90 m subsurface), so microplastics encountered by fish in the water column could be sinking due to high-specific density, fouling, or upwelling from seabed disturbances; or,

(ii) Fish were caught at different times of their DVM cycle. DVMs appear closely related to fish following prey to the surface to feed. Most published material suggests that feeding mainly occurs in upper layers at night, with lower levels of food observed in stomachs during the day (e.g. Clarke, 1978). It is likely that fish caught during the day at greater depths had fed earlier than those caught in shallower waters, and that the gut contents found during the day could be the remains from the previous night's feeding.

Food remains could have come from recent feeding or from undigested material. For example, crustacea will presumably take longer to digest than soft bodied prey and their presence in stomachs could show slow digestion rather than active feeding (Clarke, 1978; Roe and Badcock, 1984). As the analysis method dissolved digestive tracts whole, an assessment of stomach fullness or stage of digestion could not be made. Furthermore, the time from last meal and subsequent gut retention time cannot be identified as digestion rates of mesopelagic fish are unknown. Microplastics found in the digestive tract could suggest that plastics are retained following ingestion, but without knowledge of the digestion time for the study species, this is speculative.

Alternative routes of exposure

Exposure of fish to microplastic occurs primarily through ingestion resulting from targeting as food, incidental capture, being mistaken for prey, or from ingestion of prey items already containing microplastics (Lusher, 2015). Oceanic midwater fish are considered to be opportunistic feeders (Clarke, 1978) and could actively, but mistakenly, target microplastics resembling prey items. Myctophids are adapted to consume active prey. They generally have large mouths with few serrated gillrakers, well-developed stomachs, and short intestines (Beamish *et al.*, 1999). If plastics resemble prey items, mesopelagic fish may target them. Alternatively, feeding in the water column would not prevent ingestion of inorganic material and may lead to higher ingestion rates when compared with individually selected and targeted prey items.

While prey items could not be directly observed in this study, previous research describes the feeding preferences of the study species (Supplementary Information 2). Specifically focusing on the three most commonly caught species, the main prey species of *M. muelleri* are copepods, Euphausiids, and other crustacea (e.g. Gjøsæter, 1981; Young and Blaber, 1986). Diet may be related to DVM (Young and Blaber, 1986), size of organisms (Giske *et al.*, 1990), and time of year (Gjøsæter, 1981). Feeding in *B. glaciale*, which target copepods, follows similar patterns. Prey selection may be size-dependent (e.g. Giske *et al.*, 1990) and related to food availability (e.g. Gjøsæter, 1973). *Benthosema glaciale* feed cyclically at different times of the day, in relation to prey availability (Roe and Badcock, 1984), and have been observed carrying out DVM (e.g. Gjøsæter, 1973). Hence, a difference was observed between the numbers of plastics in stomachs at different times of the day, although some daytime feeding at depths has been observed (e.g. Dypvik *et al.*, 2012). No information is available on feeding preferences of *N. kroyeri*, despite the species being a primary constituent of Northeast Atlantic mesopelagic fauna. It is likely that fish selectively feed on crustacea. In fact, dietary analysis for this genus found copepods, ostracods, Euphausiids and amphipods as prey for *Notoscopelus japonicus* (Uchikawa *et al.*, 2002).

The range of species examined in this study would suggest that the most mesopelagic fish have a high potential for interaction with microplastics primarily during feeding in surface waters. Microplastics may come from eating prey. Zooplankton are the main diet of mesopelagic fish, and laboratory studies and *in situ* studies have shown microplastic ingestion by zooplankton (Cole *et al.*, 2013; Desforges *et al.*, 2015). While plastic could possibly have come from the ingestion of prey species with plastic in their gut, the median length of microplastics in this study (1.9 mm) was probably too large to be ingested by copepods and Euphausiids known to ingest particles <816 μm (Cole *et al.*, 2013; Desforges *et al.*, 2015). However, there may be underestimation in size of particles as this study only collected items >250 μm.

Potential effects of microplastic ingestion

It was not possible to determine the effects of ingestion in this study as the ability of fish to egest plastic was unknown, as was the propensity for plastic retention by the fish. Regardless of exposure, understanding the fate of ingested plastics is of primary concern, specifically for species which have important ecological roles in marine foodwebs. Microplastics may be removed through stomach evacuation, egested with undigested debris, or retained within organisms for an extended period. Interpreting the effects of ingestion is limited without knowledge of microplastic retention within the organisms.

Planktivorous fish from the North Pacific were estimated to consume roughly 12 000–24 000 tonnes of plastic per annum (Davison and Asch, 2011). This considerable amount of plastic could have a number of effects on individuals as well as the trophic system. However, there have been no reported negative effects of microplastics on wild organisms. Microplastics could be retained or egested in a similar way to invertebrates [see Lusher (2015)], they may also affect buoyancy (Boerger *et al.*, 2010), and studies have alluded to the potential implications of chemical pollutants associated with microplastics [for review, see Rochman, (2015)]. In fact, laboratory studies have shown liver toxicity (Rochman *et al.*, 2013), behavioural changes (Browne *et al.*, 2008), and endocrine disruption (Teuten *et al.*, 2009) of organisms exposed to microplastics.

Furthermore, population effects including reduced fecundity, lower survival rate, and transfer within the food chain may be related to microplastic ingestion. Empirical work is required to measure population effects before, during, and after exposure. Other marine organisms may be exposed to microplastics following egestion of faecal pellets sinking to the deep sea (Hidaka *et al.*, 2001).

If plastics are retained within mesopelagic fish, either lodged in the digestive tract or through slow passage, there is a potential for trophic level effects. Although the incidence of interaction between mesopelagic fish and microplastics is low, mesopelagic fish subsequently ingested by larger marine organisms could transfer microplastics, even temporarily, to secondary consumers (Eriksson and Burton, 2003). For example, stranded baleen whales (Besseling *et al.*, 2015) and odontocetes (Lusher *et al.*, 2015) have been found with microplastics in their digestive tracts. It was suggested that baleen whales were exposed through directly ingesting plastics from surface waters when feeding on pelagic prey, or through secondary consumption of contaminated prey, which would also be an exposure pathway for odontocetes. Mesopelagic fish are prey of odontocetes including striped dolphins and common dolphins. They make up a considerable proportion of their diet in the North Atlantic (Table 3). Mesopelagic fish have also been found in the diets of seabirds (e.g. Danielsen *et al.*, 2010) and larger fish (e.g. Olaso *et al.*, 2005). Surface feeders will not be the only organisms at risk to exposure through secondary consumption, as transport through the water column inside the fish exposes deeper marine organisms to plastics, such as beaked whales (Lusher *et al.*, 2015) and predatory squid (Braid *et al.*, 2012).

Additionally, the diel migratory behaviour of fish could provide an important ancillary pathway for microplastic distribution to the deep ocean. If microplastics pass through the digestive system, they will be released as a component of faecal matter in the deeper ocean. It is estimated that a single myctophid could transport 52 mg of food to the deep pelagic layer, and excrete 8.4 mg of faeces daily (Radchenko, 2007). Whether microplastics are retained and transported to deeper water, or ingested at depths, highlights a potential link for the exposure of a relatively unexposed ecosystem to marine pollution.

Mesopelagic fish play an important role in the biological carbon pump (Davison *et al.*, 2013) and may subsidize the metabolic demands of the deep sea. The biological pump involves active transport of organic and inorganic matter, from the euphotic zone to depths, by vertically migrating organisms (Ducklow *et al.*, 2001), where they metabolize carbon, respire (Longhurst *et al.*, 1990), egest organic carbon (Steinberg *et al.*, 2000) and faecal pellets (Wotton and Malmqvist, 2001), are eaten by other organisms or otherwise die (Zhang and Dam, 1997). Given that myctophids (lanternfish) are the dominant vertically migrating taxa and account for the greatest proportion of fish in the euphotic zone at night, they have the potential to export significant amounts of organic and inorganic carbon (Table 4) as well as microplastics to the deep sea. Myctophids are estimated to be responsible for the active transport of equivalent of 15–28% of passively sinking particulate organic carbon in the Pacific (Hidaka *et al.*, 2001; Davison *et al.*, 2013), whereas in the Mid-Atlantic Ridge area of the North Atlantic, 8% is mediated by myctophids (Hudson *et al.*, 2014). Myctophids faecal matter is a potential food resource for deep sea organisms (Hidaka *et al.*, 2001) and could thus be acting as a route of microplastic transport to organisms living and feeding in the deep sea and the benthos.

Table 3. Odontocete dietary analysis.

Species	*n*	Locations	Mesopelagic fish prey by number (%)	Reference
SD	30	Porcupine bank	65	Hernández-Milián (2014)
SD	60	Bay of Biscay	49	Ringelstein *et al.* (2006)
SD	32	Bay of Biscay	3	Spitz *et al.* (2006)
CD	63	Porcupine bank	90	Pusineri *et al.* (2007)
CD	129	Porcupine bank	54	Brophy *et al.* (2009)

SD, striped dolphins (*Stenella coeruleoalba*); CD, common dolphin (*Delphinus delphis*).

Table 4. Active transport of carbon by vertically migrating fish.

Location	Carbon export (mg C m^{-2} d^{-1})	Reference
Northeast Atlantic	0.10–0.60	Angel and Pugh (2000)
West Equatorial Pacific	8.35–24.00	Hidaka *et al.* (2001)
Mid-Atlantic ridge:		
Azorean Zone	0.40–2.78	Hudson *et al.* (2014)
Reykjanes Ridges	0.04–0.26	Hudson *et al.* (2014)
Northeast Pacific	23.90	Davison *et al.* (2013)

Calculation of the potential number of plastics consumed by marine mammals through trophic transfer

Knowledge of the percentage of mesopelagic fish containing microplastic enables estimation of the potential annual microplastic transfer to marine mammals. A calculation based on prey abundances can be made through consideration of the diets of striped dolphins by-caught in Irish waters, whose annual food consumption (as a population, $n = 88\,807$ individuals) is estimated as 16 200 tonnes of prey (Hernández-Milián, 2014). The same dietary study found that mesopelagic fish accounted for 64.8% of prey by number, such that ~0.11 tonnes of mesopelagic fish could be eaten annually by a single dolphin. This study reported that 11% of mesopelagic fish contained microplastics, which would equate to 0.012 tonnes of prey for a single dolphin. This equates to roughly ~385 million individual fish containing microplastics. As there was an average of 1.2 pieces of microplastic per fish, ~463 million microplastics could be consumed by a single striped dolphin from Irish waters through ingesting contaminated prey.

Using the same method, the levels of ingestion for individual prey species were estimated (Table 5). Assuming that the sample of 761 fish is representative of the whole mesopelagic fish community in the Northeast Atlantic, the striped dolphin population in the Irish waters ($n = 88807$, Hernández-Milián, 2014) could be exposed annually to ~42 trillion pieces of microplastic from the consumption of mesopelagic prey (Table 5).

Table 5. Trophic level consumption of microplastics by striped dolphins (*Stenella coeruleoalba*) from the North Atlantic: for (A) an individual and (B) the Irish population.

Species code	Fish consumed annually (tonnes)	Fish with plastic (tonnes)	Estimated number of individuals with plastic (million)	Plastic estimated to be consumed annually through fish (million pieces)
(A) Individual striped dolphin				
BHG	~0.05	~0.011	~272	~4000
LAX	~0.01	~0.002	~26	~28
MAV	$\sim 3.7 \times 10^{-4}$	$\sim 1.03 \times 10^{-5}$	~9	~10
Total mesopelagic fish	~0.114	~0.012	~386	~463
(B) Irish striped dolphins population				
BHG	~4568	~1005	~251	~377
LAX	~1282	~187	~2	~2
MAV	~34	~1	~0.8	~0.9
Total mesopelagic fish	~10 497	~1155	~36	~42

Calculations are based on the most commonly caught species in this study. Annual food consumption of by-caught individuals from Hernández-Milián (2014). The percentage of fish with plastic and annual consumption calculated from values in Table 2. The number of individuals calculated from species average weight. Species codes BHG—*Benthosema glaciale*; LAX—*Notoscopelus kroyeri*; MAV—*Maurolicus muelleri*.

Consequences of microplastics transfer between trophic levels

Microplastic ingestion by mesopelagic fish may have bigger consequences on secondary consumers, rather than the fish themselves. Unlike fish digestion which appears fast (dos Santos and Jobling, 1992), the digestive tracts of marine mammals have many chambers, folds, and reticulations which could increase the potential for microplastics to become lodged and retained. Studies of the passage rate of grey seals (*Halichoerus grypus*, F. 1791) found that after 88 h, the recovery rate of otoliths from scat was 98% (Grellier and Hammond, 2006). Interestingly, the same feeding study found that polystyrene beads (3 mm) were all recovered following 6-d exposure experiments (99.8%), suggesting that, although they have a long passage time, microplastics are egested with faeces. A similar observation was made for fur seals (Eriksson and Burton, 2003). Therefore, microplastics may not be retained in marine mammals. However, pinnipeds only have one chamber with folds similar to the first stomach of a dolphin (which has four chambers; Eastman and Coalson, 1974; Mead, 2007) and the digestion time may not be representative for cetaceans. Hence, as digestion takes longer in mammals, there could be a greater chance of chemical assimilation from ingestion of micro- as well as macroplastic items. While chemical transfer from microplastics to large baleen whales has been suggested (Fossi *et al.*, 2012), direct observations of ingestion and retention of plastics are required; current data from ingestion are limited to a few stranded individuals (Besseling *et al.*, 2015; Lusher *et al.*, 2015).

Conclusion

Although low in number, microplastics were found in 11% of individuals in this study. As no clear difference emerged in microplastic ingestion between species, time of day, or vertical migration patterns, microplastics may have considerable implications on the mesopelagic community and related trophic systems. Potential hypotheses to explain the route of microplastic ingestion to and from mesopelagic fish could suppose that mesopelagic fish:

(i) feed in the surface waters at night, and incidentally ingest microplastics;
(ii) selectively target microplastics and ingest them;
(iii) feed on prey that they themselves contain microplastics;
(iv) containing microplastics are themselves eaten as prey by larger predatory species, including marine mammals; and
(v) egest microplastics and faecal pellets are ingested or sink to the seabed.

The potential worldwide plastic load (1.2×10^{13} individuals with microplastic at any given time) highlights a key area for future research. The results presented here provide preliminary estimations of possible microplastic loading in mesopelagic fish communities in the Northeast Atlantic rather than an absolute measure. Further work is required to examine a range of populations throughout the globe, because mesopelagic fish are an important link between the euphotic and the aphotic zone, and could expose deep sea organisms to microplastics. While initial calculations suggest that trophic transfer could mediate transfer of large quantities of microplastics to marine mammals, microplastic retention within the receiving organisms is unknown and requires further research. If mesopelagic fish and marine mammals can egest microplastics, the exposure effects will be limited.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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