The effects of trophic transfer and environmental factors on microplastic uptake by Plaice, *Pleuronectes plastessa*, and Spider Crab, *Maja Squinado*

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Highlights

- *M. squinado* (42.5%) and *P. plastessa* (50%) sampled from the Celtic Sea Contained microplastic
- The proportion of contaminated individuals varied between site and species
- Microplastic abundance in spider crab and plaice was not linked to local fishing intensity
- Observations of microplastic in ingested sand eels demonstrate ongoing trophic transfer

Abstract

Microplastic pollution is apparent throughout the marine environment from deep ocean sediments to coastal habitats. Most of this is believed to originate on land, although marine activities, such as fishing and shipping, also contribute to the release and redistribution of microplastic. The relative importance of these maritime plastic sources, the manner by which they are distributed in the environment, and their effect on uptake by marine organisms are yet to be fully quantified. In this study, the relative impact of fishing activities on microplastic uptake by demersal fish and crustaceans was explored. Local fishing intensity, proximity to land and mean water velocity are compared to microplastic uptake in plaice, *Pleuronectes platessa*, and spider crab, *Maja squinado*, from the Celtic Sea. Observations were also made of microplastic contamination in ingested sand eels, *Ammodytes tobianus*, to establish a potential route of trophic transfer. This study is the first to identify microplastic contamination in spider crab and to document trophic transfer in the wild. Individuals were sampled from sites of varied fishing intensity in the Celtic Sea, and their stomach contents examined for the presence of microplastic. Contamination was observed in 50% of *P. platessa*, 42.4% of *M. squinado*, and 44.4% of *A. tobianus*. Locations of highest plastic abundance varied between *P. platessa and M. squinado*, indicating that different factors influence the uptake of microplastic in these two taxa. No significant link was observed between fishing effort and microplastic abundance; however, proximity to land was linked to increased abundance in *M. squinado* and Observations of whole prey demonstrate ongoing trophic transfer from *A. tobianus* to *P. platessa*. The lack of significant difference in microplastic abundance between predator and prey suggests that microplastic is not retained by *P. platessa*.

Keywords: fishing; pollution; plastic; particles; food web; sand eel

Capsule: Observations of microplastic uptake by plaice and spider crab in UK waters reveals ongoing trophic transfer from sand eels and compares the relative importance of fisheries and land based sources

Introduction

Microplastics (plastic particles measuring below 5mm) can be found throughout the marine environment. From planktonic organisms (Cole et al., 2013; Desforges et al., 2015) to top predators (Alomar and Deudero, 2017), microplastic uptake has been recorded in a variety of marine taxa representing all trophic levels and feeding modes (Cole et al., 2011). Despite the diversity of organisms seen to consume plastic, the route by which it enters the food chain is still uncertain. Whilst studies have shown a number of species are unable to distinguish between microplastics and prey items (Bern, 1990; Hämer et al., 2014) , it is unclear as to whether microplastic uptake is predominantly direct (from sea water or sediment) or indirect (for example from contaminated prey).

Both laboratory and field studies have previously been employed to explore the uptake of microplastic (Lusher et al., 2017a). Crustaceans including filtering planktonic copepods and larvae (Cole et al., 2013), isopods (*Idotea emarginata*) (Hämer et al., 2014), and decapods (Brennecke et al., 2015; Farrell and Nelson, 2013; Watts et al., 2014) have each been shown to ingest and aggregate microplastics. Observations of ingested particles have indicated that potential for aggregation in the foregut (Welden and Cowie, 2016b) and translocation into tissues (Farrell and Nelson, 2013). Microplastics may also be taken in during ventilation of the gills (Watts et al., 2014). The tendency for crustaceans to take in plastic is supported by a number of observations in wild caught animals. Whilst the number of studies is comparatively low, the uptake of microplastics in wild crustaceans has been seen to be highly heterogeneous, varying with location (Devriese et al., 2015; Welden and Cowie, 2016a). This may be partially due to variation in environmental levels of microplastic, however, retention in *Nephrops norvegicus* has also been linked to size, sex and moult stage (Welden and Cowie, 2016a).

Fewer laboratory studies have analysed the uptake of microplastics by fish, however, ingestion has been seen in a number of species (Batel et al., 2016; Lusher et al., 2017b; Mazurais et al., 2015). Wild-caught fish are more widely studied, and microplastic contamination has been reported in species from both benthic and pelagic habitats. Carnivorous pelagic fishes (Foekema et al., 2013; Lusher et al., 2013; Romeo et al., 2015; Rummel et al., 2016), demersal feeders (Lusher et al., 2013; Rummel et al., 2016), and secondary consumers have all been seen to consume microplastic. Some, such as the Japanese anchovy (*Engraulis japonicus*) may represent a trophic link to predatory species (Tanaka and Takada, 2016).

The uptake of microplastics has been shown to have negative impacts on both invertebrates and fish. Previous observations of the impact of microplastic uptake have included translocation from the stomach and gills to other tissues (Batel et al., 2016; Farrell and Nelson, 2013) and reduced nutrient uptake resulting from false satiation and nutrient dilution (Welden and Cowie, 2016b). Secondary effects include reduced reproductive success and chemical transfer (Rochman et al., 2013), and histological changes in the intestine (Pedà et al., 2016) and liver (Lu et al., 2016). In reducing the size, health and fecundity of individuals, these impacts can negatively affect the profitability and sustainability fisheries (Froese, 2004; Howarth et al., 2014).

Whether or not microplastics are transferred to humans through their food, and the potential health effects of this transfer, has also recently become a key research priority (Galloway, 2015; Rochman et al., 2015; Van Cauwenberghe and Janssen, 2014). Microplastics have been observed in several commercially harvested species of fish and shellfish including cod, haddock, mackerel (Foekema et al., 2013; Murphy et al., 2017), langoustine (Welden and Cowie, 2016a), oysters (Green, 2016), and mussels (Li et al., 2016), as well as other species from fish markets around the world (Miranda and de Carvalho-Souza, 2016; Rochman et al., 2015). If microplastics do generate negative health effects in humans, it is highly likely that these effects will increase in relation with the abundance of microplastic in our food.

The uptake of microplastic by wild caught organisms has previously been related to the scale and proximity of sources, local bathymetry and transfer from prey. As the weathering of in-use and abandoned, lost and discarded fishing gear can be a source of microplastics in the marine environment, it has been hypothesised that areas of high fishing activity will demonstrate in locally elevated levels of microplastic contamination. For the reasons above, we investigated levels of microplastic contamination in two commercially important species in the Celtic Sea to test if: (1) these organisms contained traces of microplastics; (2) whether microplastic contamination increased with levels of fishing effort or other environmental drivers; and (3) whether these organisms had become contaminated through trophic transfer. To observe the difference in plastic uptake by invertebrates and fish, this study compares two species of commercial interest; plaice (*Pleuronectes plastessa*) and spider crab (*Maja squinado*). Additional observations of microplastics ingested by the sand-eel (*Ammodytes tobianus*) were made to establish the potential importance of this species as a route of trophic transfer.

Of the two focal species, *P. platessa* is a predatory demersal flat fish which feeds on worms, molluscs and small crustaceans (Millner et al., 2005). Thanks to their wide distribution, they are targeted by European otter and beam trawlers throughout the North East Atlantic (Dunn and Pawson, 2002). In contrast, *M. squinado* is an omnivorous crustacean which can feed opportunistically on a range of food items including seaweed, detritus, invertebrates and carrion (Bernárdez et al., 2000). Due to their life history, they are only seasonally targeted by potting vessels (González-Gurriarán et al., 2002). Whilst both species routinely feed in benthic habitats and should be exposed to a similar level of environmental microplastics, crabs and lobsters, including *M. sqinado*, have a complex filter system in the gut including a hardened gastric mill which could lead to increased microplastic retention (Welden and Cowie, 2016a).

Methods

Study Site

The Celtic Sea is the region of coastal shelf bordered by the Irish Sea, English Channel and Atlantic Ocean. It reaches depths of up to 200m and contains a combination of sandy and muddy sediments. Fisheries operating in the area target a range of species including gadoids, flatfish and crustaceans. Potential sources of terrestrial plastic include the catchment of the River Severn, and industrial activities around Cardiff, Newport and Bristol. Net transport of this plastic is expected to be offshore, moving east to west.

Pleuronectes platessa and *M. squinado* were sampled from six sites in the Celtic Sea (Figure 1), chosen for similar benthic substrate and variable fishing intensity. Fishing intensity was evaluated as the swept-area ratio per year. The ratio is calculated as the mean number of times a 1.8 km^2 square is affected by trawling gear. For this study, the mean swept area was derived from data recorded by Eigaard et al (2016). In addition, the mean horizontal velocity (m s^{-1}), distance to land (m) and primary production (mg C m^{-2} yr^{-1}) were also calculated from existing GIS layers. The average annual primary production at each site was determined using MODIS satellite sensor data collected between 2009 and 2013 by NEODAAS (www.neodaas.ac.uk), at a resolution of 1.1 km2. Daily mean horizontal velocity was extracted from the data derived from the North West Shelf Reanalysis CMEMS (www.marine.copernicus.eu).

Sampling

Both *P. platessa* and *M. squinado* were collected using a 4m beam trawl with a 50mm net, deployed for 30 minutes at each site. Initial dissection was conducted onboard, and the digestive tracts were individually preserved in formalin. Removal of the digestive tract of *M. squinado* was carried out as outlined in Welden and Cowie (2016a); each individual was sexed and measured for carapace length and width, following which there carapace was cracked and the oesophagus and hind gut cut away, keeping the stomach intact until analysis. Stomachs of *P. platessa* were removed as described in Lusher et al. (2013); the length and weight of the individual were recorded, and the stomach was excised by separating at the oesophagus and midgut.

Microplastic extraction and analysis

Stomach contents were sorted under dissecting microscope at between x7.5 and x25 magnification. Potential plastics were identified using a combination of by colour, regularity of shape, surface texture, ductility and resistance to breaking. Largest fibres and fragments were removed using forceps, those too small to handle in this manner were transferred to a clean filter paper using a pipette. Identifiable prey remains were recorded and whole *A. tobianus* were individually dissected. Suspected microplastics were transferred to microcentrifuge tubes prior to confirmation of polymer composition. Whilst procedural blanks were not deployed during the initial dissection, petri dishes containing filter papers dampened with de-ionized water were used to determine the potential for contamination by ambient airborne microplastic levels during the analysis.

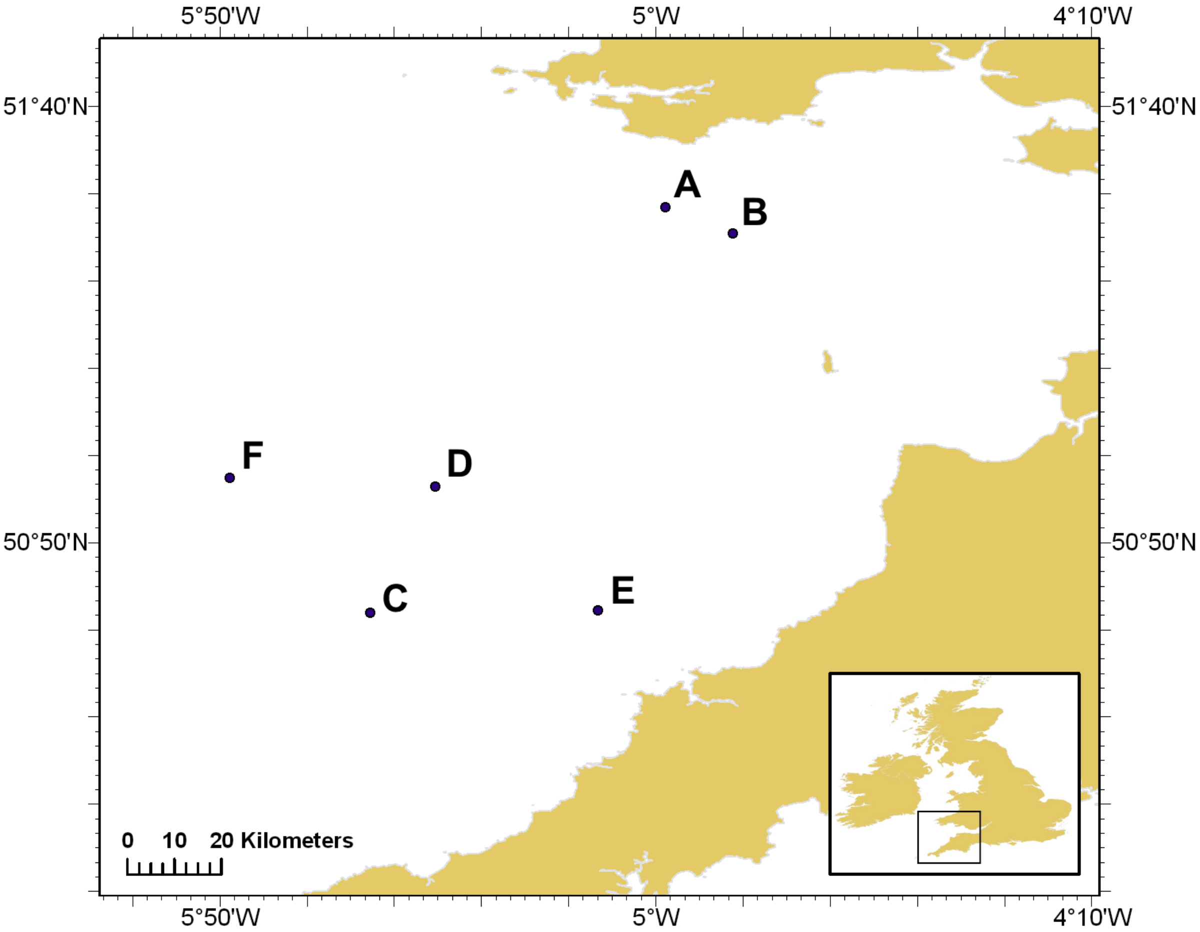

Figure 1. Sampling sites in the Celtic Sea: ***A****, 51° 28' 29.0532'' N, 4° 58' 56.1144'' W;* ***B****, 51° 25' 28.254'' N, 4° 51' 11.34'' W;* ***C****, 50° 41' 57.0444''* N, 5° 32' 48.8436'' W*;* ***D****, 50° 56' 24.9756'' N, 5° 25' 18.5772'' W;* ***E****, 50° 42' 10.4508'' N, 5° 6' 41.2308'' W;* ***F****, 50° 57' 27.4356'' N, 5° 48' 52.8408'' W.*

Fourier Transformed Infrared Spectrometry (FT-IR) was carried out on half of the samples to verify the identity of the suspected microplastics. Samples were selected for FT-IR analysis using a random number generator. Analysis was carried out at wavelengths between 800 and 4000 cm^{-1} using a Thermo Nicolet Nexus FT-IR spectrometer with attached Continuµm IR microscope. The abundance of the remaining suspected plastics was rounded down by the number of miss-identified samples.

Statistical analysis

Statistical analysis of the results was carried out as outlined in Welden and Cowie (2016a). Data were analysed using R Studio version 1.0.44. Prior to analysis, the mean number and deviation of microplastics per individual was calculated. A Kolmogrov-Smirnov analysis was used to assess the distribution of the data for each species, after which generalised linear models (GLM) were used examine the variation in microplastic in the focal species. In the first model, microplastic uptake in *P. platessa* was examined in relation to fishing intensity, primary production, mean velocity, distance from land, and fish weight. A second GLM compared microplastic uptake in *M. squinado* in relation to fishing intensity, primary production, mean velocity, and distance from land, as well as to the carapace length and sex of the individual. To produce the best model for plastic abundance in analysis with intercorrelated independent variables were examined in separate GLMs, after which stepwise reduction and lowest AIC were used to select the most relevant result.

Results

In total 140 potential microplastics were recovered, 76 of which were successfully analysed using FT-IR. Of these 12 (15.7%) were found to be misidentified. It was hoped that more than 50% of the sample would be analysed, however, the model of FTIR microscope available proved unable to provide consistent readings for smallest samples. The minimum reliable size varied in relation to the dimensions of the sample, for example fragments produced more consistent results at low dimensions than thin fibres. The level of plastics observed in the procedural blanks averaged less than 2 MP per petri dish per hour.

Gut content analysis revealed microplastic contamination in 50% of *P. platessa* and 42.5% *M. squinado* (Table 1). Microplastic was identified in *P. platessa* recovered from all sites at which they were sampled, whereas *M. squinado* from site C did not contain microplastic. *P. platessa* exhibited the highest rates of microplastic contamination at site B, whilst *M. squinado* exhibited highest rates of microplastic contamination at A and B, with 73% and 67% occurrence respectively (Figure 2). The shells of *M. squinado* were also covered with plastic fibres similar to those from fishing nets and trawl chafers (Figure 3). In both species, the most frequently ingested plastics were fibres, and when subjected to micro-FTIR analysis, the most commonly identified plastics were polypropylene, polyester, polyamide. The variation in the proportion of microplastic contaminated individuals at each site was highest in *M. squinado*, whilst the variation in microplastic uptake per individual at each site was highest in *P. platessa*.

Identifiable prey remains were similar in both focal species. Observed taxa were predominantly crustacean, small bivalves and polychaete worms. In addition to invertebrate prey, nine intact sand eels, *A. tobianus*, were recovered the foreguts of *P. platessa*, with up to seven individuals recorded in the stomach of a single *P. platessa*. Dissection and analysis of *A. tobianus* revealed that 44.4% percent contained microplastic particles (Table 1). A Mann-Whitney test was used to analyse the variation in microplastic abundance between *P. platessa* and their *A. tobianus* prey, however, no significant difference was identified ($W = 6523$, $P < 0.6835$). Prey commonly identified in the stomachs of *A. tobianus* were crustacean larvae and copepods.

Table 1. The abundance of microplastic recovered in Pleuronectes platessa*,* Maja squinado*, and* Ammodytes tobianus

Species	N	Number of contaminated individuals	Number of microplastics	Mean number of microplastics per animal (SD)
Pleuronectes platessa	109	54	79	1.46 (1.02)
Maja squinado	54	23	32	1.39 (0.79)
Ammodytes tobianus	9	4	7	1.75 (0.83)

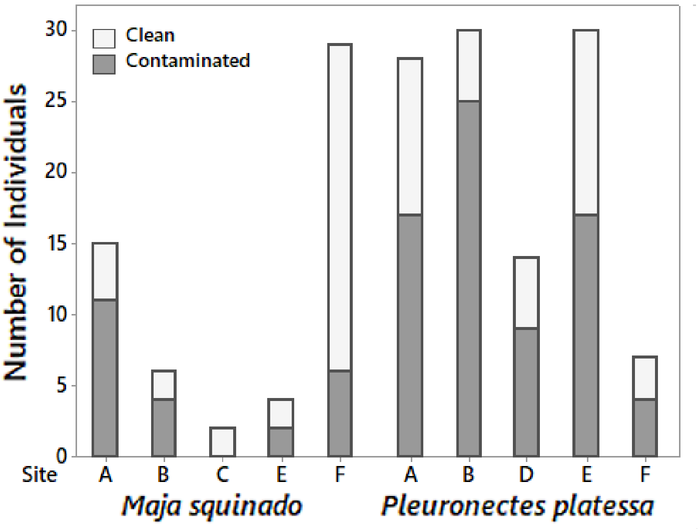

Figure 2. Plastic uptake by Pleuronectes platessa *and* Maja squinado *at each sampling site*

Figure 3. Macroplastic fibres adhered to the carapace of Maja squinado

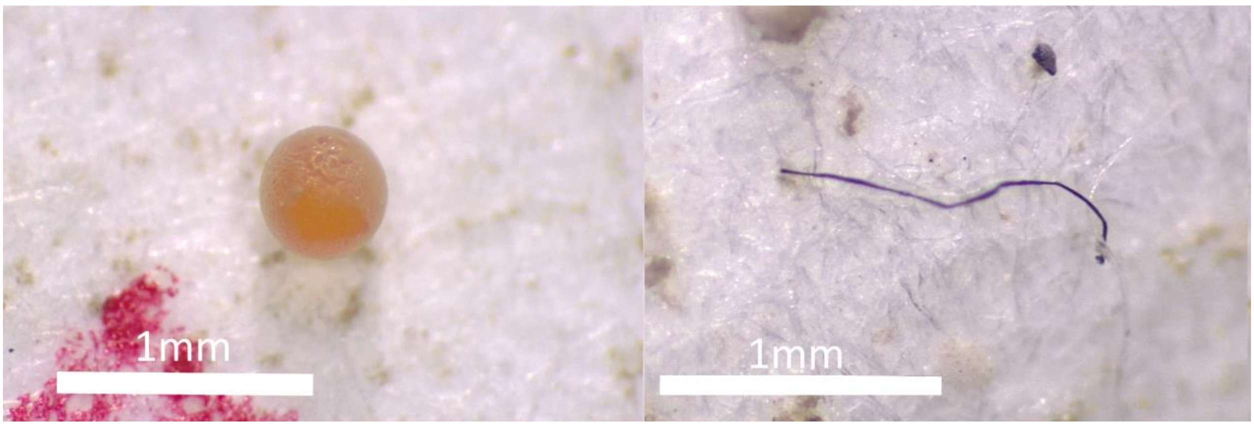

Figure 4. Microplastic pellet and fibre recovered from M. squinado (site A and F respectively)

GLM analysis of the factors significantly correlated to the uptake of microplastic by *P. platessa* and *M. squinado* revealed differing relationships between the two species (Table 2). In *P. platessa*, the abundance of microplastic was positively correlated with weight and daily mean velocity (Figure 5), and negatively correlated with primary production (Table 2). Analysis of the factors responsible for microplastic uptake in *M. squinado* revealed that only distance to land influenced the level of contamination, with animals nearshore found to contain microplastic at higher abundances (Figure 5). Notably, fishing intensity was not found to be significant in either of the analyses.

Table 2. Observed relationships between microplastic abundance and dependent variables

P. platessa

	Estimate	St. Error	T - value	P
Intercept	*1.037e+00*	*4.951e-01*	*2.094*	*0.038648*
Distance from land	1.235e-05	6.953e-06	1.777	0.078537
Mean daily velocity	2.984e+00	1.255e+00	2.378	**0.019236**
Primary Production	-3.754e-03	1.062e-03	-3.536	**0.000608**
Weight	5.249e-03	1.214e-03	4.324	**3.52e-05**

M. squinado

	Estimate	St. Error	T - value	P
Intercept	*1.243e+00*	*1.945e-01*	*6.389*	*4.65e-08*
Distance from land	-1.385e-05	3.608e-06	-3.840	**0.000336**

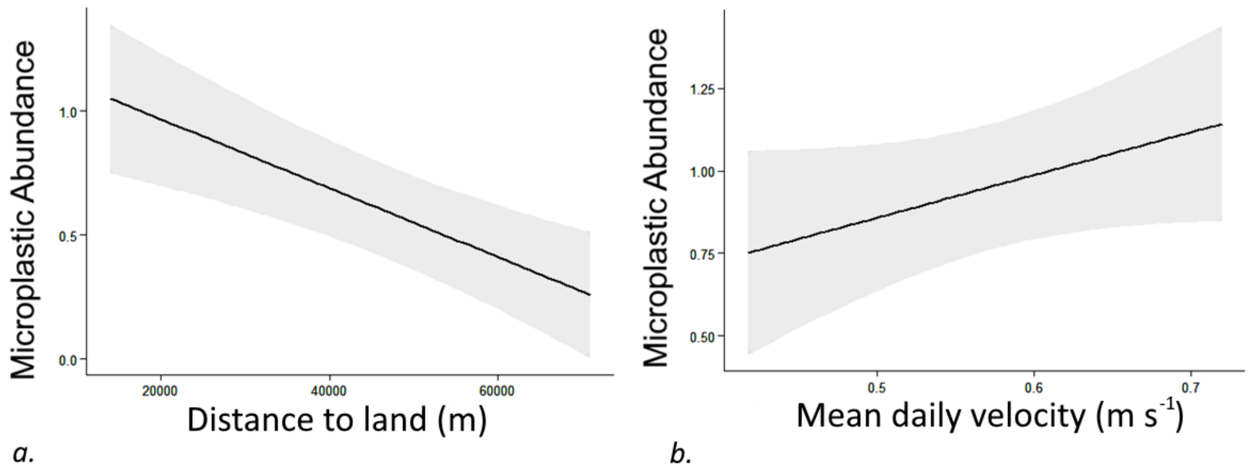

Figure 5. The statistical relationship between microplastic abundance and distance from land in M. squinado *(a) and microplastic abundance and mean daily velocity in* P. platessa *(b).*

Discussion

Microplastics were recorded at all sample sites; however, there was variation in the proportion of contaminated individuals. It is worth noting that mechanical sorting of samples limited the minimum size of plastic selected to approximately 500µm. As a result the smallest microplastic fractions may be under represented.

The carapaces of many *M. squinado* were covered in fibres, presumably as a result of contact with fishing nets or accidental addition as masking material (Parapar et al., 1997). However, high external contamination of macroplastic did not correspond to increased levels of microplastic ingestion; the maximum number of microplastics recovered per individual was 3, with an average of 1.39 items per individual. The proportion of *M. squindao* seen to contain microplastic and the abundance of microplastics recovered falls between that observed in similarly sized wild-caught crustaceans, *N. norvegicus*, from remote fishing grounds off the coast of North Scotland and those observed in the highly impacted Clyde Sea Area (Welden and Cowie, 2016a). The level of spatial heterogeneity recorded in *M. squinado* and the low level of microplastics per individual is also similar to that of the brown shrimp, *Crangon crangon*, from the southern North Sea and Channel (1.23 ± 0.99 microplastics per individual) (Devriese et al., 2015).

Analysis of the occurrence of microplastic in *P. platessa* revealed contamination in 50% of the animals sampled; however, previous studies have revealed levels of microplastic contamination far lower than those recorded here. The degree of uptake varies greatly, for example, 5.5% in Rummel et al. (2016), 11% in Lusher et al. (2015), 29% by Murphy et al. (2017), and 35% in Boerger et al. (2010). Many of the lowest occurrences of microplastic have been recorded in fish caught in offshore and mid-water trawls. Due to such extreme variation between locations and target species, comparisons between the results presented above and existing studies have been limited to those that examine nearshore environments and similar demersal feeders.

The percentage of contaminated *P. platessa* recorded here is commensurate with other studies of microplastic uptake by fish in UK waters. Whilst the abundance of fish seen to have consumed plastic is higher than that of demersal species reported from the nearby English Channel (35%) (Lusher et al., 2013), it is similar to that observed in fish in nearshore Scottish waters (45%) (Murphy et al., 2017), and below that of flounder, *Platichthys flesus*, in the Thames Estuary (McGoran et al., 2017). The average number of microplastics per animal (1.46) was also similar to that seen around the UK; higher than that recorded by Murphy et al (2017) (0.9 ± 1.79), but similar to that observed in demersal fish studied by Lusher et al. (2013) (1.2 ± 0.54).

The difference in the level of microplastic uptake and retention in *M. squinado* and *P. plastessa* is not unexpected due to the distinct morphology and feeding modes of the two taxa. Indeed, similar variation between species with different feeding modes has been observed in species found at >2200m in the Rockall Trough (Courtene-Jones et al., 2017). In addition to feeding mode, age or body mass may also affect the dimensions and number of microplastics that are ingested and retained. For example, body mass has previously been linked to lower microplastic uptake in *N. norvegicus* (Welden and Cowie, 2016a); however, this is driven by the presence of the gastric mill, a structure not found in fish. In our studied species, microplastic uptake may be affected by numerous factors linked to body size. In *P. platessa,* the relationship between length and stomach volume is a linear one, and it is predicted that a greater weight of food is consumed by larger individuals (Jobling, 1980). Increased food consumption in larger fish may result in the higher microplastic uptake seen in the statistical analysis. In *M. squinado* dietary composition may vary with age (Bernárdez et al., 2000), altering the level of microplastic to which individuals of different ages are exposed, however, in this study there was no significant link between microplastic abundance and carapace size.

Variation in plastic retention may also be related to rates of gastric evacuation or regurgitation. In *N. norvegicus,* plastics have been evacuated with the stomach lining at ecdysis (Welden and Cowie, 2016a). Many spider crabs, including *M. squindao*, do not moult after reaching sexual maturity (González-Gurriarán et al., 1995). Species which no longer undergo ecdysis must rely on microplastics being sufficiently small or appropriately oriented to pass through the gastric mill. The period between pre-pubertal moults in *M. squinado* is also highly variable (Corgos et al., 2007), the regularity with which juveniles may expel any retained plastics will have a further impact on variation in microplastic contamination in this species.

The apparent differences in microplastic uptake between the two focal species indicate that observations of high microplastic abundance in one species cannot be used to infer high contamination throughout a community. Whilst this variation between taxa is expected, the significance of these results will be determined by whether the relationship between the microplastic uptake rates of the two species remains consistent across a range of environmental microplastic concentrations. Consistent relationships between species may allow extrapolation of contamination between species, reducing the level of potentially damaging sampling required.

The Effect of Site Specific Factors and Local Fishing Intensity on Microplastic Uptake

The abundance of recovered microplastic in both *M. squinado* and *P. platessa* varied between the six sampled sites; however no common factors influencing microplastic abundance were found between the two species. In addition to the different factors affecting microplastic abundance, *M. squinado* exhibited higher variation between locations.

The composition of recovered polymers indicates a probable combination of land based and fisheries sourced plastics. Local fishing pressure may affect microplastic availability and uptake in a number of ways, either by introducing microplastic via the weathering of fishing gear or by the resuspension of deposited plastics during the disturbance of seedbed sediments (Churchill, 1989). However, statistical analysis indicated no significant links between the abundance of microplastics and fishing intensity. In addition to being a potential source of microplastics, trawling results in the resuspension of sediment. Plumes of sediment and microplastics are re-distributed as a result of the tidal state, circulation and wind patterns apparent during the resuspension event (Floderus and Pihl, 1990). As a result, environmental factors may mediate and diffuse microplastic inputs from trawling.

It is probable that microplastic uptake by benthic and demersal species in coastal environments is driven by proximity to shore and land based sources. This is supported by the significantly higher levels of microplastics in *M. squinado* at nearshore sites. This is particularly apparent at site A and B and is also visible in *P. platessa* at site B, the two nearshore sites. Similar observations have been made in *N. norvegicus* in Scottish coastal waters, in which microplastic abundance was highest in the nearshore site (Welden and Cowie, 2016a).

In *P. platessa*, microplastic abundance was positively correlated with the mean daily water velocity and primary production. The reduction in microplastic abundance per individual at sites of high primary production may be the result of local increases in population density. At sites of high primary production there may be increases in the abundance of primary consumers and predatory species (Frederiksen et al., 2006). Assuming a similar level of microplastic between locations, the higher density of feeding animals may result in fewer microplastics available to an individual. However, as we are not able to definitively state the level of microplastic in the water column a great deal of additional observation is needed for this explanation to be accepted.

The higher average microplastic abundance in *P. platessa* has also been linked to areas of increased water velocity. Faster currents may result in the refloatation and reduced deposition of microplastic. For example, it is known that increased water movement during storm events results in higher concentrations of suspended plastic (Lattin et al., 2004), and periods of elevated wave activity have been linked to increases in the mean abundance and mean size of microplastics (Reisser et al., 2015). Elevated levels of microplastic at the water-sediment interface may be the source of increased microplastic uptake in these fish.

Trophic Transfer to P. platessa

Observation of microplastic in the stomach content of ingested *A. tobianus* indicates an active route of trophic transfer to *P. platessa*. *A. tobianus* are predominantly plankton feeders, and microplastic may be taken up from seawater or from copepods and other zooplankton, a group known to ingest microplastic (Desforges et al., 2015). *Ammodytes* sp. are a key prey species for many piscivorous organisms (Frederiksen et al., 2006; O'Connell and Fives, 1995). Although the number of individuals sampled in this study is small, there is clear evidence that predatory fish, seabirds and marine mammals are at risk of microplastic uptake via trophic transfer (Furness, 2002; Rindorf et al., 2000). A similar observation has been made in captive common seals, *Halichoerus grypus*, fed wild caught mackerel, *Scomber scombrus*; in which microplastics were found in 32% of the fish analysed and 48% of seal scat subsamples (Nelms et al., 2018).

In addition the presence of *Ammodytes* sp., previous dietary observations of *P. platessa* have revealed polychaetes such as *Pectinaria* and *Nereis*, bivalves including *Ensis and Spisula*, and crustaceans including *Upoebia* and *Macropipus* (Rijnsdorp and Vingerhoed, 2001). Their diet is temporally variable; the relative importance of polychaete prey is seasonal, with the weight recorded increasing to over 60% of the stomach contents in summer months. Similarly, larger individuals have a reduced dependence on annelids, increasing their consumption of bivalves, echinoderms and vertebrate prey (Rijnsdorp and Vingerhoed, 2001). Polychaetes, such as *Nereis* (Lourenço et al., 2017) and *Arenicola* (Van Cauwenberghe et al., 2015) have also been seen to take in plastics in the wild, and may represent a further route of transfer to plaice; however, this could not be confirmed in the current analysis due to the highly degraded state of soft bodied prey.

Mann-Whitney analysis comparing the abundance of plastic in *A. tobianus* and *P. platessa* species revealed no significant difference in plastic loads between the trophic levels. This indicates that whilst *P. platessa* may consume multiple individuals containing microplastic, most will be readily egested and not retained in the gut. A positive relationship was observed between microplastic abundance and individual weight in *P. platessa*. As indicated in the previous section, this may be the result of increased feeding rates by larger individuals, with a greater number of contaminated prey items being consumed in a shorter time period.

Impacts of microplastic ingestion on Maja squinado *and* Plueronectes platessa

Ingestion of microplastics may have a range of effects on the health of our focal species. Retention of plastics by crustaceans has been seen to result in aggregation in the gut, reduced feeding and lower nutritional state (Blarer and Burkhardt-Holm, 2016; Watts et al., 2015; Welden and Cowie, 2016b). Microplastics at the lower end of the size range may also translocate into the tissues (Brennecke et al., 2015; Farrell and Nelson, 2013), resulting in a range of physiological effects such as reduced mobility and survivorship (Tosetto et al., 2016). However, these effects may not be apparent in animals which do not contain large aggregations of microplastic or are not exposed for extended periods (Hämer et al., 2014), as observed in *Echinogammarus marinus* (Bruck and Ford, 2018) and *Uca rapax* (Imhof and Laforsch, 2016).

In fish, microplastic ingestion has been linked to translocation (Lu et al., 2016), reduced predatory performance and feeding efficiency in *Pomatoschistus microps* (de Sá et al., 2015), changes in the histology and lipid uptake in the liver of *Danio rerio* (Lu et al., 2016), and altered histology and function in the intestine of *Dicentrarchus labrax* (Pedà et al., 2016). As in crustaceans, the potential of microplastics to negatively affect an organism may be dependent on the degree of aggregation and retention time. Analysis of microplastic consumption in goldfish has suggested that particles over 63µm are not held in the gut for extended periods (Grigorakis et al., 2017). Low retention time may be responsible for lack of plastic contamination observed in a number of species; for example, eelpout, *Zoarces viviparus*, sampled from the Baltic Sea and North Sea, which were not seen to contain plastic (Wesch et al., 2016).

In addition to the potential direct impacts of microplastic uptake on *P. platessa*, there may be indirect effects related to contamination of prey species. *A. tobianus* that have ingested microplastic may have lower feeding rates and reduced nutrient assimilation similar to those outlined in the species above. As a result, microplastic contaminated prey may have lower nutritional value. Regularly consuming prey of lower quality would reduce the foraging efficiency of *P. platessa* and other predators, requiring individuals to spend a greater time foraging and feeding. As more information is generated describing the energetic and nutritional costs of microplastic uptake, so models must be developed to project these effects through the trophic levels.

Conclusions

Crustaceans and fish from the Celtic Sea were both seen take in plastic at levels commensurate with other studies of coastal waters around the UK; however, the pattern of microplastic uptake varied between the two taxa. Fishing intensity and the associated microfibres released from trawl nets did not significantly raise the level of microplastic in either species. Instead, proximity to shore resulted in a greater contamination in *M. squinado* and body weight, mean water velocity and increased primary production were linked to variation in microplastic abundance in *P. platessa*. This study is the first to confirm the trophic transfer of plastics in progress in the marine environment, raising concerns over the relative nutritional value of prey and the effect on the foraging efficiency of *P. platessa*. In addition to highlighting the traditional issue of impacts on the contaminated organism, researchers must now consider the impacts of microplastic uptake on the commercial value of economically important fish and shellfish species.

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