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Microplastic contamination in brown shrimp (*Crangon crangon*, Linnaeus 1758) from coastal waters of the Southern North Sea and Channel area

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ABSTRACT

This study assessed the capability of *Crangon crangon* (L.), an ecologically and commercially important crustacean, of consuming plastics as an opportunistic feeder. We therefore determined the microplastic content of shrimp in shallow water habitats of the Channel area and Southern part of the North Sea. Synthetic fibers ranging from 200 μm up to 1000 μm size were detected in 63% of the assessed shrimp and an average value of 0.68 ± 0.55 microplastics/g w. w. (1.23 ± 0.99 microplastics/shrimp) was obtained for shrimp in the sampled area. The assessment revealed no spatial patterns in plastic ingestion, but temporal differences were reported. The microplastic uptake was significantly higher in October compared to March. The results suggest that microplastics >20 μm are not able to translocate into the tissues.

1. Introduction

Plastic is the wonder product of this century due to their low production costs and specific characteristics like durability and flexibility. This has led to an ever growing use and demand with a year on year expansion of approximately 9% from 1950 to 2012, leading up to a plastic production of 299 M ton in 2013 (PlasticsEurope, 2013, 2014). Plastics have become an essential part of our modern lifestyle and nowadays plastic can be found in most products, from children's toys to winter jumpers. Through accidental release and thoughtless discards, the same plastic has accumulated in the marine environment. Around 70% of marine litter consists of plastic items, mainly originating from land (Barnes et al., 2009; Wright et al., 2013a). Unfortunately, the discarded bags, bottles and other waste items washed up on beaches are not the only problem. The invisible, small microscopic pieces of plastics or microplastics are likely to far outweigh the bigger litter items and pose greater risks to animals and plants (Law and Thompson, 2014). Microplastics have been defined as pieces of plastic smaller than 5 mm and can originate from direct spillage and/or breakdown of bigger plastic items (Moore, 2008; Moore et al., 2011). They can range in size from being invisible to the naked eye to just a few millimeters in diameter (Thompson et al., 2004). Pollution of the oceans by these tiny pieces of plastic debris is now so widespread, that they are turning up in all the world's major seas and oceans (Wright et al., 2013a; Eriksen et al., 2014; Woodall et al., 2014).

Microplastics ingestion and effects have been reported in a wide range of organisms including decapod crustaceans, such as the shore crab and Norway lobster (e.g. Murray and Cowie, 2011; Farrell and Nelson, 2013). Most of the effects, however, have been observed in experimental set-ups with very high microplastics values (e.g. Von Moos et al., 2012; Besseling et al., 2013; Cole et al., 2013; Watts et al., 2014). Only a rather limited number of studies investigated the ingestion of microplastics in wild organisms in their natural habitat. An overview is given in Table 1, which demonstrates the uptake of microscopic particles by organisms with diverse feeding strategies at different levels of the food-web (Murray and Cowie, 2011; Braid et al., 2012; Goldstein and

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Table 1
Microplastics ingestion by wild aquatic organisms in their natural habitat.

Aquatic organisms	Origin	Microplastics level	Organ/tissue examined	Size MP	Reference
Arenicola marina	BE, NL, FR	1.2 ± 2.8/g w. w.	Whole organism	>5 μm	Van Cauwenberghe et al. (2015)
Mytilus edulis	NL	3.5 fibers/10 g w. w.	Whole organism	>20 μm	De Witte et al. (2014)
Mytilus edulis	GE	0.36 ± 0.07/g w. w.	Whole organism	5–10 μm, 11–15 μm, 16–20 μm	Van Cauwenberghe and Janssen (2014)
Crassostrea gigas	GE	0.47 ± 0.16/g w. w.	Whole organism	21–25 μm m and >25 μm	Van Cauwenberghe and Janssen (2014)
Commercial fish	UK	1.90 ± 0.10 particles on average per individual	Gastro-intestinal tract	Unclear	Lusher et al. (2013)
Commercial fish	NL	2.6% of fish contained plastic	Gastro-intestinal tract	>0.2 mm	Foekema et al. (2013)
Stellifer brasiliensis	BRA	6.9% contained plastics	Stomach	Nylon fragments	Dantas et al. (2012)
Stellifer stellifer	BRA	9.2% contained plastic	Stomach	Nylon fragments	Dantas et al. (2012)
Nephrops norvegicus	UK	83% of animals contained plastics in stomach	Gut	<5 mm polypropylene rope	Murray and Cowie (2011)
Dosidicus gigas	CA	Plastic pellet	Stomach	<5 mm	Braid et al. (2012)
Gobio gobio	FR	12% contained plastic	Gastro-intestinal tract	Fibers and pellets	Sanchez et al. (2014)
Lepas spp.	NPSG	33.5%	Gastro-intestinal tract	Particles	Goldstein and Goodwin (2013)

Goodwin, 2013; Foekema et al., 2013; Lusher et al., 2013; Wright et al., 2013a; De Witte et al., 2014; Sanchez et al., 2014; Van Cauwenberghe and Janssen, 2014; Van Cauwenberghe et al., 2015). Mainly filter-feeders such as mussels, oysters and barnacles are investigated for microplastic uptake (Table 1). For example, the microplastics content of mussels for human consumption and wild mussels from groynes and quaysides (Belgium) varied from 2.6 to 5.1 fiber/10 g w. w. on average (De Witte et al., 2014). Van Cauwenberghe and Janssen, 2014 concluded that the exposure of European consumers to microplastics amounts to up to 11,000 plastic particles per year through the eating of shellfish. Wild crustaceans have also been assessed for microplastics content. Norway lobsters (*Nephrops norvegicus*) sampled in the Clyde Sea have been found to ingest microplastics, as 83% of the individuals sampled had plastics in their stomachs (Murray and Cowie, 2011). Most of these particles were fiber-like structures. To date, no field study has been conducted on the presence of microplastics in the most common species of shrimp in the North-East Atlantic, the brown shrimp (*Crangon crangon*).

The European brown shrimp (*C. crangon*), also known as common shrimp, is an epibenthic decapod species, which can be found in shallow coastal and estuarine soft-bottom areas such as sand and mud, where studies have shown high accumulation of microplastics (e.g. Andrady, 2011; Claessens et al., 2011; Ivar do Sul and Costa, 2014). This means that shrimp are potentially exposed to microplastics with different concentrations throughout their life-cycle. Therefore brown shrimp are considered prime suspects for further investigation in this study. Brown shrimp have a wide distributional range in the North-east Atlantic, from the White Sea in the north of Russia to the coast of Morocco in the south, and are most abundant in the North Sea, Dutch Wadden Sea, the German Bight, and the Dutch and Danish coasts (Tiews, 1970; Redant, 1984; Campos and Van der Veer, 2008). These are also the main fishing areas where more than 600 vessels from six countries (Belgium, Denmark, France, Germany, the Netherlands and the UK) target this species (Hufnagl, 2009; FAO, 2013). Consequently, shrimp is an important socio-economic species with a global capture production of almost 40,000 tons (FAO, 2014).

Due to its abundance, *C. crangon* plays an important role in the functioning of coastal shallow ecosystems. It forms an important food item for a large range of predators, such as gadoids, pleuronectids and gurnards as well as for birds and crustaceans (Henderson et al., 1992; Walter and Becker, 1997; Del Norte-Campos and Temming, 1998). In addition, *C. crangon* is an opportunistic feeder and adult shrimp consume macrofaunal species such as polychaetes (*Nereis* spp.), mollusks, small arthropods and juvenile stages of fish such as newly recruited plaice, but will also devour algae (Pihl and Rosenberg, 1984; Henderson & Homes, 1987; Kamermans and Huitema, 1994; Dolmer et al., 2001; Oh et al., 2001). It was already suggested that some of these preys are able to ingest microplastics, which underlines the potential role of shrimp in the process of trophic transfer of microplastics (e.g. Cole et al., 2013; Wright et al., 2013b). Due to temporary as well as seasonal changes in benthic communities, they must be able to cope with different prey species and thus utilize a broad and variable spectrum of diets (Teschke and Saborowski, 2005). The digestive tract of these crustaceans is complex. It is composed of the mouth parts, where the ingested material is mechanically chopped (Rojo et al., 2010). The intestine extends all along the abdomen from the posterior end of the stomach and terminates at the anus (Glass and Stark, 1994; Sánchez-Paz et al., 2007). The multipart intestinal tract could provide storage capacity or even a longer retention of microplastics after ingestion.

The aim of this study is to assess if brown shrimp in natural populations are consuming microplastics, while taking into account the crucial role *C. crangon* plays in ecosystem functions and the food web and its commercial value. Hence, spatio-temporal patterns of microplastics contamination in brown shrimp and its potential effects on the nutritional health condition of the organisms were investigated. The microplastic uptake by shrimp enables an assessment of the potential ecological and human health risks associated with the presence of these plastics.

2. Materials and methods

2.1. Study area and sampling strategy

Five institutes carried out sampling across the Channel area and Southern part of the North Sea between France, Belgium, The Netherlands and the UK by towing a shrimp trawl at receding tide (Fig. 1). Samples were collected at Oostende Oosteroever (BE), Kijkduin Den Haag (NL), Lowestoft beach (UK) and Pointe de l'Arcouest (FR). Samples were preserved in aluminum foil at

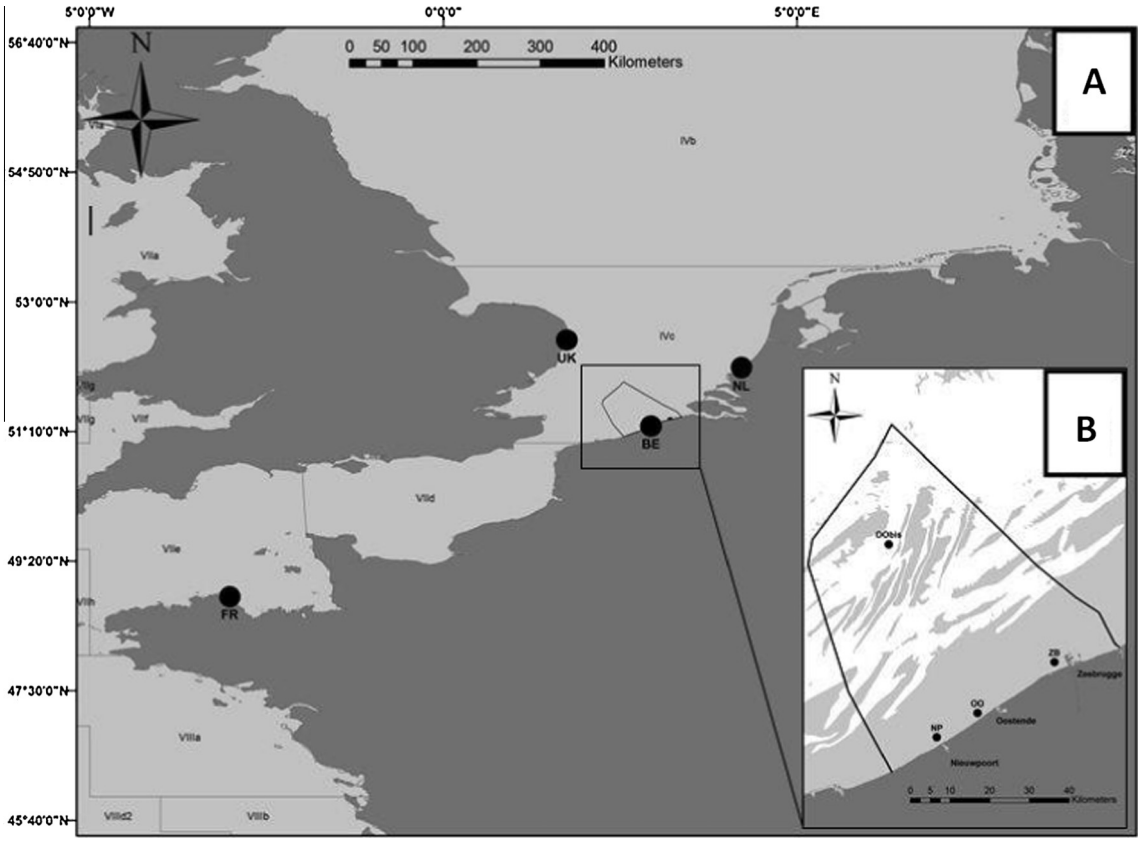

Fig. 1. (A) overview map with the 4 sampling locations in shallow water habitats of the InterReg 2 Seas and France Channel area. (B) overview map with the 4 sampling areas on the Belgian part of the North Sea (NP: Nieuwpoort, ZB: Zeebrugge, OO: Oostende or OObis: Oostende bis).

−20 °C. Only the sample from FR was preserved on ethanol (Sigma–Aldrich, 70%).

Additionally, ILVO (Belgium) sampled brown shrimp on the Belgian part of the North Sea (BPNS) between March 2013 and March 2014 using a 8 m or 4 m beam trawl net with shrimp net (22 mm mesh width). Sampling was performed during two different seasons, March (2013, 2014) and October (2013), as shrimp exhibited different nutritional health condition during these seasons. Samples were collected near the mouths of the three Belgian coastal harbours of Zeebrugge (ZB), Oostende (OO) and Nieuwpoort (NP), and on other locations further off shore of Oostende (OObis) (Fig. 1B). All shrimp were washed with filtered type 1 ultrapure water to remove the majority of sediment grains. In all cases, firstly *C. crangon* species were identified and isolated after which shrimp of 45–55 mm carapace length (eye socket to tail) were preserved in aluminum foil at −20 °C.

A total of 165 shrimp were collected from 8 different sites. Whole shrimp (including exoskeleton) ($N = 45$) and peeled shrimp (abdominal muscle tissue representing the edible part with removed digestive tract) ($N = 45$) were analyzed separately to properly investigate the external and internal microplastic contamination.

2.2. Microplastics analysis

The whole and peeled shrimp were destructed as described by De Witte et al. (2014). Rigorous precautions were adopted while handling and processing the samples to avoid airborne and solvent contamination with microplastics (De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014). The extraction of microplastics from the shrimp bodies was performed overnight, using an acid digestion with a mixture of nitric acid (VWR, 65%) and perchloric acid (VWR, 68%), HNO_3:$HClO_4$ (4:1 v:v) in a closed fume hood. The digest was boiled (>80 °C) for 10 min, followed by a dilution of the digest with 500 ml heated and filtered type 1 ultrapure water. The solution was boiled a second time until the tissue was completely digested as observed by visual inspection, followed by a cool down period of 30 min. The acid digest was filtered over a qualitative filter (VWR, Grade 310; retention of 10–20 μm) and the filter was transferred on a glass Petri dish for transport and visualization of microplastics under a stereo microscope (Leica M 20:5:1 or M 16:5:1 zoom). No tissue fraction remained on the filter after filtration of the digest, which indicates a total digestion of the shrimp tissues. Observed microplastics were classified by category (fiber – film – spherule – fragment) and color for each assessed shrimp or blank sample. A hot point test (hot needle held with tweezers) was done on suspected particles. The hot point will make the plastic sticky and leave a mark. Plastic particles were not further identified by polymer type. One destruction batch was performed for each location, which consisted of 5 shrimp and 3 procedural blank analyses. The procedural blanks were performed without tissues in parallel with samples containing biota. For the evaluation of microplastics in the tail muscle tissues of brown shrimp (edible part for human consumption), the whole shrimp were peeled and dissected to remove the digestive tract in a clean-air cabinet. The acid destruction and visual inspection were performed as described above for whole shrimp.

2.3. Morphometric correlation and condition factor

For the evaluation of the overall health condition of the caught shrimp, the length-weight relationships were compared to the

Table 2
Morphometric correlation (Log_{10} $W = a + b * Log_{10}$ CL) and the assessed number of individuals (N) for different sample collections of brown shrimps.

a	b	$r2$	N	Sample collection
−4.420	2.647	0.97	64	Robinson et al. (2010)
−5.259	3.219	0.92	45	March 2014 – BE
−4.966	3.021	0.94	50	October 2014 – BE
−2.526	1.489	0.81	5	NL – DELTARES
−5.142	3.162	0.93	10	FR – IFREMER/CNRS
−6.131	3.725	0.97	10	UK – CEFAS
−5.585	3.387	0.89	10	BE – ILVO

morphometric correlation of Robinson et al. (2010) for North Sea *C. crangon* (Table 2). The strength of length-weight relationship:

$$\mathrm{Log}_{10}W = \mathrm{a} + \mathrm{b} * \mathrm{Log}_{10}CL$$

with W as wet weight (g), CL as carapace length (mm) and a, b as regression coefficients, is determined by coefficient of determination ($r2$) and slope value (b) (Robinson et al., 2010).

The dry weight condition index (KI) based on the correlation between dry weight and carapace length is advised for the evaluation of nutritional condition of crustaceans (Pérez-Castañeda and Defeo, 2002; Perger and Temming, 2012). In this study, it was however not possible to assess the dry weight of the shrimp as the shrimp still had to be digested for microplastics content. The condition factor for each individual was therefore calculated according the Fulton's condition factor (Fulton, 1904) adapted for decapod crustaceans (Patil and Patil, 2012):

$$K = 100W/\mathrm{CL}^3$$

where K represents the condition factor, W the mean wet weight (g) and CL the mean carapace length (mm).

2.4. Data analysis

Based on the three procedural blanks, an average background value for microplastics contamination was established for each batch. Results were evaluated after blank subtraction. An average amount of microplastics per 1 g w. w. with standard deviation was reported for each destruction batch or sampling point.

Statistical treatments were done using Statistica V12.5. Previously to parametric analysis, Levene's test for homogeneity of variances and normality of data (Q–Q test on residuals) set were tested on the transformed data. The significance level used was $\alpha = 0.05$. Visualization of the transformed data set was obtained using Box–Whiskers plots. The amounts of plastic uptake by shrimp caught in BE, NL, FR and UK, as well as the differences between the plastic content of shrimp from 4 different areas in Belgium (OO, OObis, NP, ZB) were compared using parametric analysis by one-way ANOVA (multiple groups).

The dissimilarity of condition factor, length and weight between sample collections as well as the differences between the plastic content of shrimp caught in October 2013, March 2014 and October 2014 were evaluated using non-parametric analysis by Kruskall–Wallis (K–W) test for multiple independent groups. The evaluation of condition factors for 2 independent groups (with and without microplastics contamination) for each sample collection was done by t-test for independent groups.

3. Results

3.1. Morphometric correlation

For all sampling collections of shrimp, the slope value (b) was higher than the value from Robinson et al. (2010) except for the shrimp from the Dutch coast (Table 2). The deviation in morphometric correlation could be explained by the difference in weight class of these shrimp compared to the total collection. No significant deviation in weight (K–W, $p = 0.09$, df: 3), length (K–W, $p = 0.17$, df: 3) or condition factor K (K–W, $p = 0.22$, df: 3) between the coastal shrimp from BE, NL, FR and UK could be observed. Based on wet weight, a significant difference between the shrimp from the Dutch coast and the shrimp collections from October 2013 (K–W, $p = 0.02$, df: 5) and March 2014 (K–W, $p = 0.001$, df: 5) could be noticed.

As expected, a significant variation (K–W, $p = 2 \times 10^{-6}$, df: 5) was observed between the condition factor K of the shrimp collection of March 2014 and October 2013 (Table 2 and 3). The condition factor K is significantly higher for shrimp caught in March (average $K = 0.001306 \pm 0.000123$) compared to shrimp caught in October (average $K = 0.001175 \pm 0.000121$). The slope value (b) from the morphometric correlation is also larger for the shrimp from March compared to the shrimp from October, which indicates that the shrimp from March are in a better physical condition as they grow larger compared to the shrimp from October.

3.2. Mitigating background contamination

The acid extraction of microplastics from shrimp was subjected to background contamination by microscopic synthetic fibers. To overcome these limitations, strict measures were taken in the laboratory for these tissue destructions as mentioned by De Witte et al. (2014). The whole shrimp were peeled and dissected to remove the digestive tract in a clean-air cabinet to avoid airborne contamination. Airborne contamination included mainly fibers >1500 μm and was easily recognized. In addition, airborne contamination was mainly observed on the outer periphery of the filter. Only fibers <1000 μm in the inner circle of the filter were considered as potential contamination on the procedural blank filters and included in the blank subtraction. Only black, blue and red fibers were observed regularly on the filters of the blank samples, while the blank samples were completely free of plastic particles (film, granules and spherules). Orange and pink fibers were never observed in the procedural blanks, and only 1 purple fiber and 7 translucent fibers were observed on the blank filter during analysis of all the shrimp samples. This indicates that the background level of contamination seemed to be below the LOD values, specified by De Witte et al. (2014).

3.3. Microplastics content

Microscopic synthetic fibers ranging from 200 μm up to 1000 μm size were detected in 104 of the 165 (63%) individual assessed whole shrimp. The majority (96.5%) of the microplastic contamination was categorized as synthetic fiber. The shrimp ingested mainly purple-blue (43%), yellow-greenish (50%), translucent (15%) and orange (12%) fibers, but also transparent (8%) and pink (2%) fibers (Fig. 2A). Translucent fibers could be originated from colored fibers due to the acid destruction protocol. Of the 165 individually assessed whole shrimp, only 1 shrimp (0.6%) ingested a plastic granule and 8 shrimp (4.8%) ingested a piece of plastic film. Due to the particle retention of 10–20 μm on the qualitative filters microparticles smaller than the filter limit could not be considered. The observed plastic granule and pieces of plastic film were rather small with dimensions ranging between 20 μm and 100 μm. Based on the 165 analyzed whole shrimp (Table 3), an average microplastic content of 0.68 ± 0.55 microplastics/g w. w. or 1.23 ± 0.99 microplastics/shrimp based on an average weight of 1.8 g/shrimp was obtained. The standard deviation revealed high individual variation between individual shrimp on microplastic ingestion.

Table 3
Average carapace length (CL) in mm, average wet weight (w. w.) in g, average condition factor (*K*) and the average microplastics content with standard deviation (number per g w. w.), and the assessed number (*N*) of shrimps is presented for each sampling location (BE, UK, FR and NL) or area (OO: Oostende, ZB: Zeebrugge, NP: Nieuwpoort).

Sampling area	Sampling date M/Y	CL (mm)	w. w. (g)	*K*	Number of microplastics/g w.w.	Stdev	Number individuals
BE	03/14	48.2	1.34	0.0012	0.75	0.47	5[b]
UK	04/14	45.8	1.28	0.0012	1.76	1.64	5
FR	04/13	43.4	1.11	0.0013	1.21	1.75	5
NL	06/14	40.6	0.74	0.0011	0.40	0.56	5
OO	03/14	50.2	1.63	0.0013	0.44	0.39	15[b]
OObis	03/14	51.7	1.75	0.0012	0.65	0.01	10
ZB	03/14	49.1	1.67	0.0013	0.03	0.04	10
NP	03/14	54.4	2.26	0.0014	0.19	0.17	10
OO	10/13	54.0	1.90	0.0012	0.70	0.33	15
OObis	10/13	44.4	0.98	0.0011	1.92	0.61	10
ZB	10/13	53.0	1.90	0.0012	1.09	0.33	15
NP	10/13	51.7	1.79	0.0012	0.58	0.59	10
OO	03/13	45–55[a]	2.39	–	0.20	0.18	15
OObis	03/13	45–55[a]	2.53	–	0.46	0.15	15
ZB	03/13	45–55[a]	2.51	–	0.63	0.24	15
NP	03/13	45–55[a]	1.94	–	0.96	0.61	10

[a] The exact carapace length was not measured for the shrimp collection of March 2013, and consequently no information on condition factor is available.
[b] The 5 individual shrimps of Belgium (Oostende) are also included in the 15 individual shrimps of Oostende (March 2014).

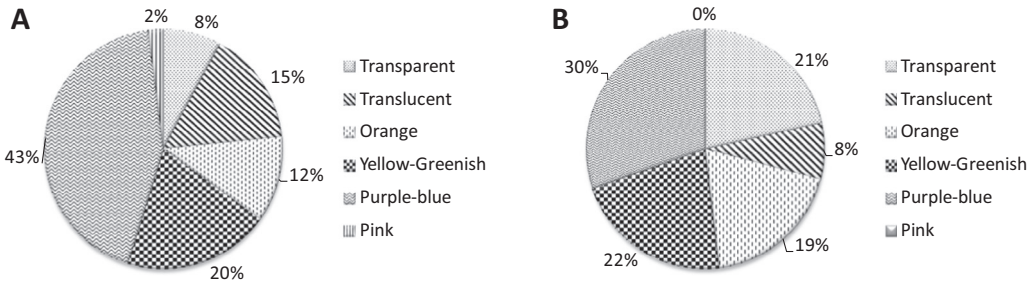

Fig. 2. Pie chart of the observed synthetic fibers in all assessed shrimp (A) and shrimp from coastal areas in BE, NL, FR and UK (B) categorized by different colors (%): transparent, translucent, orange, yellow-greenish, purple-blue and pink.

The sample collections from BE, NL, FR, UK, March 2014 and October 2013 were individually analyzed for potential correlation between microplastics contamination and condition factor, to avoid interference of other spatio-temporal factors on the evaluation of shrimp condition. The regression statistics of the relationship between the condition factor *K* and the microplastic content are provided for the shrimp collection of October 2013 (Supplementary Fig. 1) and March 2014 (Supplementary Fig. 2). This regression analysis revealed no linear relationship between condition and microplastics content for the shrimp from October 2013 ($r2$: 0.0119) as well as for the shrimp from March 2014 ($r2$: 0.1890). Subsequently, each collection was divided into two groups of shrimp, one without and one with microplastics contamination. No relationship was found between the condition of the shrimp and the level of contamination of microplastics within an individual sample collection (*t*-test, $p = 0.64$, df: 52), which indicates that microplastic contamination does not affect the nutritional condition of shrimp and reversely that the size of shrimp does not affect the microplastics ingestion based on the assumption of a positive relationship of the size of shrimp with food intake.

A piece of plastic film was observed in 4 individual whole shrimp, while no plastic particles were observed in the tissues of peeled shrimp. Although the digestive tract, shell and head were removed in a clean-air cabinet, contamination of synthetic fibers did occur in some destruction batches and procedural blank samples. For the batches without airborne contamination, no fibers were observed in the tissues of the peeled shrimp, which suggests fibers or other microplastics >20 μm are not translocated from the digestive tract into the tail muscle tissues.

3.4. Spatial patterns of microplastics contamination

The evaluation of microplastics content in whole shrimp from coastal areas in BE, NL, FR and UK revealed that 14 (70%) of the 20 assessed shrimp contained microplastics (fibers). These shrimp were not subjected to gut depuration, but were killed immediately after sampling. The average amount of microscopic fibers ranged from 0.40 (FR) to 1.76 (UK) fibers/g w. w. (Table 3, Fig. 3). The shrimp from coastal areas in BE, NL, FR and UK ingested purple-blue (30%), yellow-greenish (22%), transparent (21%) and orange (19%) and translucent (8%) fibers (Fig. 2B). Pink fibers were not observed. No other types of microplastics such as fragments or beads were observed in the evaluated shrimp. No significant (ANOVA, $p = 0.38$, df: 3) difference was obtained between the plastic content of shrimp from different locations and an average value of 1.03 fibers/g w. w. was established for *C. crangon* in the shallow water habitats along France, Belgium, Netherlands and the UK. However, the sample sizes were too low to reliably estimate a general contamination level. As these shrimp were not collected on the same sampling occasion, this could have influenced the interpretation of spatial patterns of microplastics contamination. A large inter-individual variation of microplastic contamination was observed on each location (STDEV ranging from 0.47 (BE) to 1.75 (FR)), which indicates the need to collect large sample sizes per sampling location.

Microplastic content was therefore assessed on a larger number of shrimp ($N = [10–15]$) from 4 different areas on the BPNS (Fig. 4) in March 2013, October 2013 and March 2014 No significant differences were observed between the ingestion of plastic in shrimp

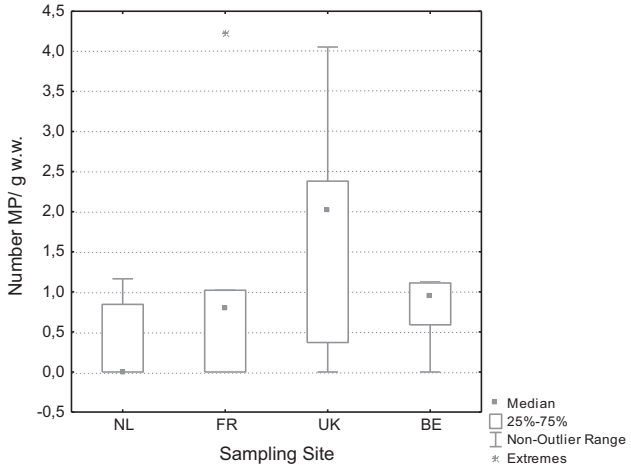

Fig. 3. Average microplastics content observed in brown shrimp from shallow water habitats of the InterReg 2 Seas and France Channel area sampled on 4 different locations (BE, UK, FR, NL).

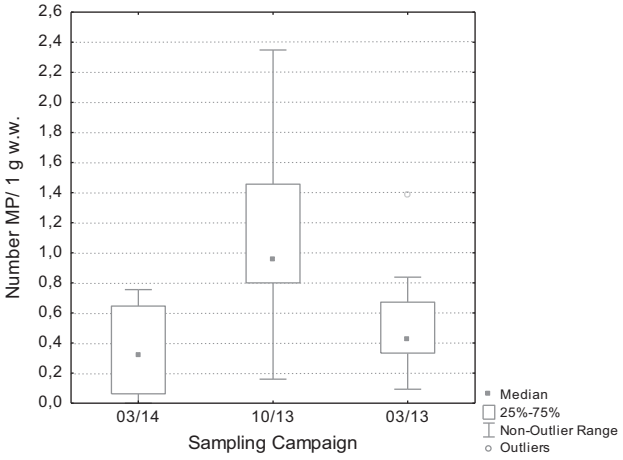

Fig. 5. Number of consumed microplastics by shrimp caught in March 2013 ($N = 55$), October 2013 ($N = 50$) and March 2014 ($N = 45$) on the BPNS.

from the 4 different areas, concerning shrimp from each sampling period or concerning the total shrimp collection ($N = 150$) on the BPNS (ANOVA, $p = 0.34$, df: 3). The highest average microplastics content (1.92 ± 0.61 microplastics/g w. w.) was observed during October 2013 in the area OObis and the lowest (0.03 ± 0.04 microplastics/g w. w.) during March 2014 in the area ZB (Table 3). An average microplastics content of 0.64 ± 0.53 microplastics/g w. w. shrimp was established for *C. crangon* on the BPNS.

3.5. Temporal patterns of microplastics contamination

As no significant differences in microplastic contamination of shrimp between areas were demonstrated, data from the different areas were combined. The evaluation of microplastics content in whole shrimp caught on the BPNS revealed an average microplastic content of 0.53 ± 0.36 microplastics/g w. w. for March 2013 ($N = 55$), 1.04 ± 0.62 for October 2013 ($N = 50$) and 0.34 ± 0.31 for March 2014 ($N = 45$) (Fig. 5). The amount of microplastics ingested by shrimp differed significantly among October 2013 and March 2013 (K–W, $p = 0.009$, df: 2) and March 2014 (K–W, $p = 2 \times 10^{-5}$, df: 2), but not between March 2013 and March 2014 (K–W, $p = 0.21$, df: 2). These results indicate a significantly higher number of consumed plastic by brown shrimp in October compared to March.

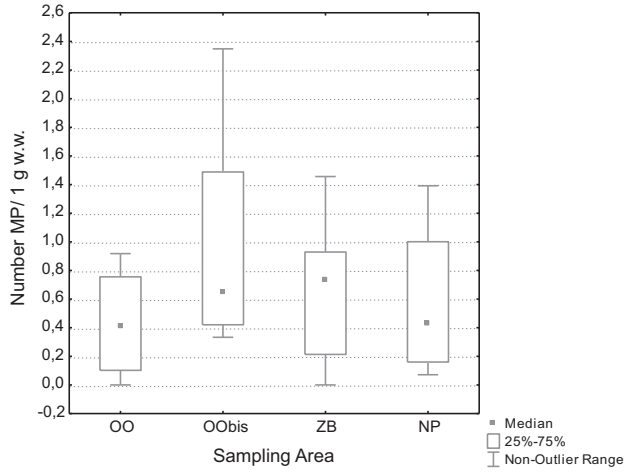

Fig. 4. Average microplastics content observed in brown shrimp from 4 areas on the BPNS (NP, OO, OObis and ZB) sampled during March 2013, October 2013 and March 2014.

4. Discussion

4.1. Microplastic uptake by brown shrimp

The results reveal that *C. crangon* is able to consume plastic and that 63% of the shrimp from the shallow water habitats of the Channel area between FR, BE, NL and UK has ingested microscopic plastic. The majority of the observed plastic particles were small monofilaments. This is in agreement with the observation of microplastic contamination in other species, e.g. *Mytilus edulis* and *N. norvegicus* (Murray and Cowie, 2011; De Witte et al., 2014) and with the observation by Claessens et al. (2011) that synthetic fibers are the most abundant type of microplastic in sediments of the Belgian Part of the North Sea (BPNS). The results in this paper indicated a rather anecdotic ingestion of other types of microplastics by brown shrimp. The level of microplastics uptake by shrimp of the BPNS in this study (0.64 ± 0.53 microplastics/g w. w.) is clearly elevated compared to the level of microplastics in a comparable amount of sediment considering the average microplastics content of 0.097 ± 0.019 microplastics/g d. w. sediment of Claessens et al. (2011). A microplastic level of 330 particles/kg dry weight on sediment from OO sampled March 2014 was reported by van der Meulen et al. (2014), consisting of 364 fibers and 66 spheres/kg dry weight sediment. Shrimp of the same sampling occasion contained on average 0.44 ± 0.39 fibers/g w. w. or roughly 440 fibers/kg shrimp. Individual shrimp of this area ingested 0.08–1.35 fiber during March 2014. Altough 20% of the observed microplastic in sediment consist of spheres, this work showed that shrimp did not ingest plastic spheres. This could be due to the discrepancy between the retention of the filter for biota (10–20 μm) and sediment (0.7 μm) analysis, as a result of which the smallest micrometer-sized ingested particles are not included. Another possibility is that shrimp have rather a preference for fibers or ingest fibers by accident and are able to avoid plastic spheres. The concept of a preferable ingestion of microplastics, based for example on the colors of plastic and consequently the food/prey resemblance is still controversial between researchers, but could here be the reason for higher uptake (e.g. Kawamura et al., 2010; Boerger et al., 2010; Schuyler et al., 2012; Verlis et al., 2013).

The present study with shrimp tissues revealed only plastic particles in the whole shrimp, but no particles in the tail muscle tissue, suggesting that particles are present in the digestive tract, the head or gills of the shrimp and not in the abdominal muscle tissue, which constitutes usually the edible part. However, translocation of microplastics between the gastro-intestinal system and tissue has been suggested for mussels with particles of 2 and 4 μm (Browne et al., 2008; Von Moos et al., 2012). Other research implied that there is an upper size limit of particles capable of being translocated from the gastrointestinal tract into the tissues (Hussain et al., 2001), which could be an important aspect for the evaluation of microplastics larger than 10–20 μm in the present study. Brown shrimp are usually peeled before human consumption, discarding the cephalothorax. However, the digestive tract is not always completely removed for this small type of shrimp and possibly the microplastics in the digestive tract could be transferred to humans during consumption. Based on the average Belgian consumption of 0.5 kg shrimp/person (Fockedey, 2006), considering a best case scenario that 90% of the microplastics will be removed by peeling, a quick indicative assessment revealed an ingestion rate between 15 and 175 microplastics each year per person. On the other hand, brown shrimp form an important food item for a large range of predators, and thus ingested microplastics may accumulate in predators higher up the food chain. Such trophic level transfer of microplastics was suggested previously by Farrell and Nelson (2013). Recently, Setälä et al. (2014) confirmed trophic transfer of 10 μm polystyrene spheres by three types of mysid shrimp using grazing experiments with zooplankton labeled with spheres.

4.2. Effects of microplastics uptake on nutritional condition

After the destruction of the shrimp, not only some microplastics, but also a noteworthy amount of sediment particles remained on the filter. These sediment particles were clearly bigger and more abundant compared to the observed microplastics in shrimp. These facts together with the knowledge on the excretion of non-digested products and sand particles from the stomach, suggest that microplastics do not accumulate in the intestinal tract or stomach of the shrimp and will be excreted together with the sand particles. For example, Setälä et al. (2014) demonstrated egestion of microspheres by mysid shrimp after 12 h. It is possible, however, that spherical and granular particles were easily removed from the digestive tract as suggested for crabs (Farrell and Nelson, 2013), while the uptake and egestion of synthetic fibers are more ambiguous and appeared to be delayed due to the complexity of the digestive tract of crustaceans. As suggested for the decapod crustacean *N. norvegicus* (Murray and Cowie, 2011) and demonstrated for spheres in shore crabs *C. maenas* (Watts et al., 2014), some of the fibers could be retained in gut and gills. However, fibrous microplastics have been less studied in laboratory trials so far and therefore it is unclear whether residence time of these particles is comparable to that of spheres (Watts et al., 2014).

As the microplastics will probably be excreted together with the sand particles, it seems unlikely that the consumed amounts of plastic will directly affect the general health condition of shrimp. This hypothesis is supported by the observation in this study that microplastic ingestion was not significantly correlated with the nutritional condition factor of shrimp. Foekema et al. (2013) did not find any significant association between the condition factor (size–weight relationship) of North Sea fish and the presence of ingested plastic particles, supporting our results in shrimp. These observations give an indication, but are definitely not sufficient to confirm the hypothesis that microplastic uptake itself has an impact on the condition of the field-collected organisms in their natural habitat at current microplastic concentrations.

4.3. Patterns of microplastics uptake

In our study, no significant spatial patterns of ingestion could be observed for shrimp, whereas Foekema et al. (2013) observed significant difference between plastic ingestion in fish from the northern North Sea compared to those from the southern North Sea. They concluded that the higher frequency of fish with ingested plastics in the southern North Sea represented higher local plastic pollution levels. The present study revealed on the other hand a significantly higher microplastic uptake by shrimp in October (2013) compared to March (2013 and 2014).

Seasonal fluctuations on the occurrence of plastic and plastic ingestion were observed in estuary environments, mainly due to the increase of freshwater and rainfall during the rainy season (Dantas et al., 2012; Lima et al., 2014). Salinity distribution showed that the water mass of the BPNS is mixed with freshwater originating mainly from the Rhine and Meuse rivers (with a smaller contribution from the Scheldt Estuary), and revealed a seasonal effect in salinity with a minimum impact during winter period (Lacroix et al., 2004). This freshwater discharge in the North Sea could also have contributed to a higher microplastics uptake found in October as compared to March. Also, particle modeling based on hydrodynamic transport processes revealed that accumulation areas of microplastics in the North Sea are around the coastal areas and floating microplastics follow the hydrodynamics to the north-east (Van der Meulen et al., 2014). A seasonal distribution of litter transport in the North Sea towards some coastal regions was suggested by the analysis of Neumann et al. (2014).

Knowing that fibers are the most common type of microplastics in the environment and depending on the color and polymer type; the fibers could originate from fisheries, recreational boating, laundry and domestic wastewater and other local human activities (e.g. Browne et al., 2011; Claessens et al., 2011). Plastic contamination in wild mussels from Belgium was assigned to degraded fishing gear such as dolly rope (De Witte et al., 2014). The use of dolly rope by the Flemish fishermen was recently rated on 133,000 kg/year (Bekaert et al., 2014). On the BPNS, shrimp beam trawling is the most important fishing activity and the majority of the vessels are trawling within the coastal 3 miles area (Pecceu et al., 2014), covering the sampling area of this study. The highest shrimp landings are reported during summer time, even until October and November, while the lowest landings are observed during the period January–April (Van Hoey et al., 2014). The elevated fishing activity during October compared to March could be responsible for the higher (micro)plastic availability to shrimp.

In addition to the fluctuations in presence of microplastics in the environment, shrimp are more active in October building fat reserves to prepare for winter time. This implicates that shrimp will take up more food and possibly associated microplastics, which may be one of the potential explanations for the higher occurrence of microplastics in October. The results of the morphometric correlation and the condition factor indicate a beneficial health condition in March compared to October, not related to the individual microplastics uptake. Variation in condition and growth rates of brown shrimp may vary considerably due to changes in water temperature, salinity, food availability and foraging behavior, reproductive cycle, molt events and intermolt period (e.g. Boddeke, 1982; Paschke et al., 2004; Delbare et al., 2014).

4.4. Shrimp as a key species in environmental monitoring

Assessment of microplastics in sediments is currently ambiguous, time consuming and expensive and it provides no information on the bioavailability of the microplastics for marine life. Therefore, the evaluation of microplastics in key species may reflect the impact of microplastics on a specific area. Based on

the JAMP Guidelines for Monitoring Contaminants in Biota (OSPAR, 2010) some basic prerequisites and criteria should be considered for the selection of key species to be monitored for microplastic contamination. In this context, it is important that species such as shrimp are easy to sample and strong enough to survive under laboratory conditions for effect assessment. Shrimp were already used as indicator organisms for TBT and PCBs in the North Sea (Roose et al., 1998; Verhaegen et al., 2012); and according to Roose et al. (1998), shrimp are excellent indicator organisms which clearly reflect the quality status of their habitats. If shrimp are used as monitoring tool for microplastics in the environment, the question arises if the consumed amount of microplastics reflects the microplastics level in the sediment. As mentioned earlier, the consumed amount of plastic by shrimp is high compared to the actual level in the sediment. At the moment, no correlation with environmental microplastic levels could be established. In addition, the ingestion of microplastics seems to vary extremely between the individual shrimp, even between shrimp sampled at the same time on the same location. As the destruction protocol described in this paper is rather time-consuming, it would be recommended to use pooled samples (e.g. 25–100 shrimp) from a chosen target population with specified length-range, area and time. This way, the results will reflect changes in the concentration of microplastics in the surrounding environment and could be integrated in environmental monitoring programmes and contaminant databases. For the evaluation of microplastic contamination in biota, attention must be drawn to the challenges of harmonization of research and monitoring procedures, integrated assessments and quality assurance (implementation of interlaboratory exercises, procedural blanks, detection limits). According to the advice of the International Council for the Exploration of the Sea (ICES, 2015), the choice of which plastic to monitor in biota is related to the technical ability to monitor within biota. The method for microplastic evaluation proposed in this work applies a cut-off size of 20 μm, whereby only plastic larger than 20 μm is observed. Plastics can occur in several shapes, but synthetic microfibers are particularly difficult to monitor as they are pervasive throughout the environment and the lab. For this reason, mitigation of airborne fibers is needed and procedural blanks are required. Acids such as HNO_3 and $HClO_4$ involve a good digestion of the tissues and other organic material, leaving only silica (e.g. sand particles) and plastic particles. Further studies are required on the use of concentrated HNO_3 or $HClO_4$ as there are reports of detrimental effects on fibers of nylon (Claessens et al., 2013; ICES, 2015).

5. Conclusion

The results of this study clearly indicate that shrimp are able to ingest microplastics, especially synthetic fibers. An average microplastics content of 0.68 ± 0.55 microplastics/g w. w. or 1.23 ± 0.99 microplastics/shrimp was obtained for shrimp in the Channel area and Southern part of the North Sea. Although no spatial pattern was observed, a clear seasonal effect on the microplastic contamination in shrimp was established. The higher uptake in October compared to March could be assigned to the feeding rate of shrimp, the influence of freshwater or fishing activities in the area. The results also suggest that particles >20 μm are not able to translocate into the tissues. However, transfer of microparticles to humans by eating brown shrimps without removing the intestinal tract cannot be ruled out. Lastly, no negative effect of microplastics ingestion on the nutritional condition of shrimp could be observed. However, collateral damage induced by microplastics ingestion, due to the availability of chemicals or bacteria present on the plastic particles, was not investigated in this study and could outline a potential health concern.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2015.06.051.

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