Ingested microplastic is not correlated to HOC concentrations in Baltic Sea herring

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Abstract

Plastic debris and microscopic particles (MP) are ubiquitous in our waters, and can be ingested by various aquatic organisms. Ingestion of MP has been linked to decreased food intake and bioaccumulation of organic contaminants carried by MP. However, field data on MP ingestion and linkages between MP and contaminant levels in biota are limited. We examined occurrence of plastic particles in the stomachs of herring (*Clupea harengus membras*) collected along the Baltic west coast in concert with environmental contaminant concentrations in fish muscle. We found that MP are common in fish stomachs and their quantity varies geographically. Approximately 50% of the fish examined had ingested plastics, which is ~10-fold greater than what has previously been reported for herring in the southern Baltic Sea. The median number of particles per fish was however, low (0-1 per sea basin). Moreover, the number of MP in the stomachs decreased with age and reproductive stage but increased with body size. Contaminant concentrations in the muscle tissue were not related to MP occurrence in the stomachs, suggesting no connection between the bioaccumulation of contaminants and MP ingestion. Thus, although present ubiquitously, MP contribute only a minor part of the diet and are not likely to measurably contribute to the total body burden in the Baltic herring.

Keywords: *Microplastic, Baltic Sea, herring, hydrophobic organic contaminants, marine monitoring*

1. Introduction

Ingestion of plastic debris, and, in particular, the smaller size fraction termed microplastic (MP < 5 mm), has been recorded in a wide range of both marine and fresh water animals across multiple trophic levels (Lusher *et al*. 2013, 2015, Cole *et al*. 2013). Concerns regarding the ingestion of MP by commercial fish and shellfish species have been particularly alarming due to the direct link to human consumption and the potential effects of MP on health (EFSA 2016). This has led to an increasing number of studies aiming to quantify MP-ingestion in fish (Lusher *et al*. 2013, Foekema *et al*. 2013, Rummel *et al*. 2016).

In various animals, the ingestion of microplastics has been associated with decreased food intake (Cole *et al*. 2015, Ogonowski *et al*. 2016) and bioaccumulation of hydrophobic organic chemicals (HOCs) (Oliveira *et al*. 2012, Besseling *et al*. 2013, Rochman *et al*. 2013b) that effectively sorb to plastics (Rochman *et al*. 2013a). Modelling studies have also indicated that plastics may act as passive samplers once ingested (Herzke *et al*. 2016, Koelmans *et al*. 2016), thereby decreasing the body burden of hydrophobic chemicals. Environmental data linking the body burden of HOCs and ingested MP by biota is, however, limited.

In this study, we provide quantitative data on MP ingested by Baltic Sea herring (*Clupea harengus membras* L.) which is a facultative, pelagic filter-feeder (Huse and Toresen 1996) with a high risk of being exposed to MP. The samples were taken along the Swedish east coast, from the Bothnian Bay in the north to the Bornholm basin (Hanö Bight) in the south, covering approximately 1500 km of the coastline. To assess whether MP significantly contribute to HOC body burden in the fish we also investigated the relationship between internalized plastic and muscle tissue concentrations of HOCs that were measured in the same fish specimens within Swedish Marine Environmental Monitoring program.

2. Materials and Methods

2.1 Fish collection and sample characteristics

Baltic herring for analysis were obtained from the Swedish Museum of Natural History. The fish were sampled along the Swedish coast as a part of the Swedish National Monitoring Program for Contaminants in Marine Biota. In total, 130 specimens were randomly selected from thirteen monitoring stations and used for MP analysis (Figure 1). These stations were chosen to reflect the representative range of HOC levels on a regional scale and not to be exposed to point sources.

Approximately 50% of the fish were females and the sex ratio was uniform across the samples. The fish had an average total length of 17.3 ± 1.8 cm and were ~ 4-5 years old. The reproductive phase, as determined by gametocytic maturity, varied widely geographically, ranging from immature to mature. The entire gastrointestinal tract (GIT) was removed, packed in aluminum foil, frozen at -20 °C, and stored until MP –analysis, that was conducted at the Department of Environmental Science and Analytical Chemistry, Stockholm University, Sweden.

a. *Plastic quantification in fish stomachs*

Individual GITs were placed in a Petri dish, dissected with chirurgical scissors, and washed with deionized particle-free water. Each sample was visually inspected under a stereo microscope and any

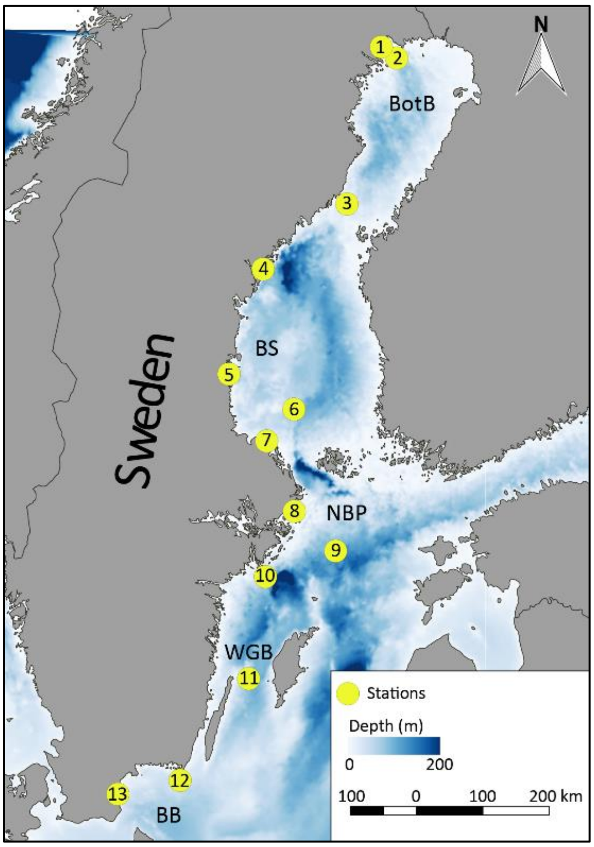

Figure 1. Sampling sites within the Swedish National Monitoring Program for Contaminants in Marine Biota included in this study, BotB Bothnian Bay, BS Bothnian Sea, NBP Northern Baltic Proper, WGB Western Gotland Basin and BB Bornholm Basin. 1 Rånefjärden, 2 Harufjärden, 3 Holmöarna, 4 Gaviksfjärden, 5 Långvindsfjärden, 6 Bothnian Sea offshore site, 7 Ängsskärsklubb, 8 Lagnö, 9 Baltic proper offshore site, 10 Landsort, 11 Byxelkrok, 12 Utlängan and 13 Western Hanö bight.

bolus items resembling plastic were extracted using stainless steel pincers, counted and transferred to Eppendorf tubes for further analysis. To account for variable feeding rates and corresponding variability in the amount of ingested plastic, we normalized the plastic counts per stomach using the gut fullness weighed by the mass of the fish, calculated as: gut fullness (%) × body weight (g wet weight). Gut fullness was categorized on a semi-quantitative scale as 0% (empty), 25%, 50%, 75% or 100% (full); stomach volume was assumed proportional to body weight (Pirhonen and Koskela 2005).

Criteria for the visual identification of MP followed recommendations of Norén (2007) and (Hidalgo-Ruz *et al*. 2012) and were as follows: (i) color, particularly bright and unnatural colors as well as same color over the whole length of a particle or a fiber, (ii) no organic structures, and (iii) uniform diameter over the whole length of a fiber. Each potential plastic particle was categorized according to its shape (fiber or fragment) and color. Particles smaller than 1 mm were not considered. Particles > 5 mm were recorded but not used for further analyses.

2.3 Controls and blanks

To control for potential contamination of airborne particles, the dissections were performed under a Fumex local extractor and each sample was studied for 10 minutes. As a blank, a Petri dish filled with deionized water was placed next to each sample and used to quantify and characterize background contamination. A cotton lab coat and nitrile gloves were used at all times to avoid contamination by clothing, and use of synthetic garments was restricted. The type and color of clothing was also recorded for each dissection event in order to back-trace potential contamination. If present, samples that would display quantifiable amounts of background contamination (plastic particles > 1 mm) were excluded.

2.4 Chemical analysis

Fish muscle samples for contaminant analysis were taken from the middle dorsal muscle layer. For individual fish, 10 g of muscle was used, whereas 1 g per individual was used for pooled samples (n=10). Tissue sampling was performed according to standard procedures (TemaNord 1995). The samples were analyzed for HOCs such as polychlorinated biphenyls (PCB 28, 52, 101, 153 and 180), organochlorine pesticides (DDEK, DDDK, DDTK, HCB and Lindane) , HCHs (AHCH and BHCH) and polybrominated flame retardants (BDE 28, 47, 99, 100, 153 and 154 and HBCD) according to the guidelines of the Swedish National Monitoring Program for Contaminants in Marine Biota. Details of the analytical procedures are given elsewhere (Bignert *et al*. 2016).

2.5 Statistical analysis

Prior to statistical analyses, all values below the limit of quantification (LOQ) were replaced by the LOQ divided by the square root of 2. To assess the degree of correlation between variables, we performed a factor analysis using the sum of contaminants within their respective chemical groups and plastic abundance in the GIT. Differences between stations and basins were tested using Permanova (Anderson 2001), while relationships between specific variables were tested using generalized additive models (GAM). Due to an overrepresentation of zeros in the data, the models were run using zero-inflated Poisson error structures. All analyses were performed in R 3.3.2 (R Core Team 2014).

3. Results

3.1 Microplastic concentrations in the GIT

Microplastic particles identified by visual inspection were found in 68 out of the 130 analyzed fish (50.4%), with a median abundance of one plastic fiber and/or fragment (range: 0-53) per individual. Among the microplastics recovered, fibers dominated (87.6%), while fragments were less frequent (12.4%). The variation in total plastic concentrations between stations and basins was high (Table **1**), and we did not find any significant difference in the normalized abundance of MP among the basins (*station* nested within *basin* as a random factor, pseudo $F_{4,117}$=1.254, p = 0.33).

The procedural blanks all contained plastic to some extent (mainly fibers). The particles were however all < 1 mm and did thus not measurably contribute to the counts. No samples were discarded due to contamination.

Table 1. Range (min-max) of the microplastics recovered from fish guts. The data are shown by particle shape and color (median = 0 for all single, per basin categories) as well as total MP content (median) and range (min-max) in the gastrointestinal tract of Baltic herring. Values are given per basin and ordered north to south.

	Fiber					Fragment				
Basin	**Black**	**Red**	**Brown**	**Green**	**Translucent**	**Black**	**Red**	**Green**	**Median MP content**	**Range (min-max)**
Bothnian Bay	0-7	0-38	0-4	0-0	0-3	0-0	0-3	0-12	1	0-40
Bothnian Sea	0-8	0-18	0-0	0-0	0-5	0-7	0-0	0-2	1	0-27
Northern Baltic Proper	0-1	0-0	0-0	0-0	0-51	0-0	0-2	0-2	0	0-53
Western Gotland Basin	0-1	0-3	0-0	0-1	0-1	0-0	0-7	0-0	0	0-7
Bornholm Basin	0-1	0-13	0-0	0-0	0-2	0-0	0-0	0-0	1	0-13
Total	**0-8**	**0-38**	**0-4**	**0-1**	**0-51**	**0-7**	**0-7**	**0-12**	**1**	**0-53**

3.2 Linkage between ingested plastic and HOCs

The factor analysis revealed that the abundance of ingested MP in herring stomachs was unrelated to the concentration of HOCs (Figure 2). Microplastic occurrence in GIT displayed a significant negative loading on the first axis and positive loading on the second. The organochlorine pesticides loaded strongly only on the first axis while the PCBs and PBDEs displayed some degree of positive loading on both axes.

3.3 Biological factors related to the amount of ingested plastic

Age and reproductive phase were generally negatively related to the MP occurrence in the GIT (GAM, χ^2 = 13.3, p < 0.001 for *age* and χ^2 = 36.9, p < 0.0001 for *reproductive phase*), where plastic content decreased in fish older than five years of age. This also correlated positively with *reproductive phase*. *Gut fullness* displayed a bell shaped relationship with plastic content where maximal amounts of plastic in fish with half-full guts were found. Albeit significant (GAM, χ^2 = 34.0, p < 0.0001), this relationship was not particularly strong and likely of low biological importance. The body weight was not related to the amount of plastic for smaller fish but showed a strong positive effect in fish > ~50 g (GAM, χ^2 = 69.4, p < 0.0001).

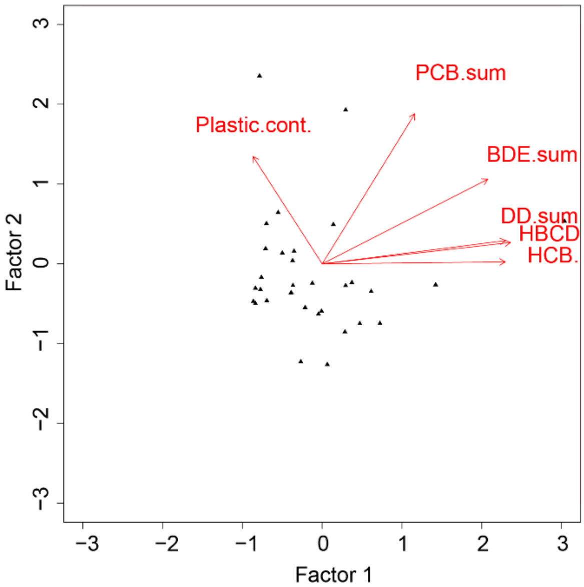

Figure 2. Factor scores (axes) and loadings (arrows) of contaminants (HBCD, HCB and the sum of PCBs, BDEs and DDs) and average plastic content (*Plastic.cont*)

Discussion

4.1 High prevalence of microplastics in the GIT

The frequency of microplastics in the gastrointestinal tract was relatively high (~50%), with a high contribution of fibers (88 %), similar to values reported by Lusher *et al*. (2013) from the English Channel (36.5% containing MP out of which 68% were fibers). However, Foekema *et al*. (2013) and Rummel *et al*. (2016) reported a much lower overall prevalence of fibers (up to 10%) in the fish collected in the North Sea and the Southern Baltic Sea. Moreover, they found only up to 2% of the herring containing plastics. Other possible reasons for the discrepancy between our results and those of Foekema's and Rummel's can be related to the size and gut fullness of the fish examined. Indeed, Foekema and co-workers analyzed considerably larger fish that probably already had switched from planktonic filtering to raptorial feeding (Huse and Toresen 1996). This may improve selection against zooplankton-sized plastic particles. Other selective feeders, such as copepods can also avoid ingesting MP (Cole *et al*. 2011). In the study by Rummel and co-workers, the fish stomachs were empty, indicating arrested feeding related to spawning. This agrees well with our observations that the amount of internalized plastic increased with fish size (gut volume) and decreased with reproductive phase. Since the absolute amount of ingestable particles, intuitively should scale with both fish size and gut fullness, we expected to see a similar pattern for the gut fullness as for the body weight. This was however not the case. One explanation is that the rate of plastic egestion is slower compared to natural food resulting in a non-linear response; such effects have been observed with plastic fibers in amphipods (Au *et al*. 2015). Alternatively, the observed bell shaped response with maximum plastic ingestion at intermediate gut fullness could be an effect of variable feeding rates, related to varying levels of hunger.

The bell shaped but weak response curve for *gut fullness* indicates that normalizing the internalized plastic content by this variable does not measurably contribute to the standardization of internalized gut content. Fish size on the other hand, seems to have a stronger effect on the absolute amount of ingested particles and should be used to standardize internalized MP concentrations. However, ontogentic changes in feeding modes or behavior as well as maturity levels should also be considered.

4.2 Absence of correlation between plastics and HOCs

Although the so called ”Trojan horse” effect (Cole *et al*. 2011), i.e., transfer of hydrophobic contaminants from plastic to biota has been demonstrated under laboratory conditions (Rochman *et al*. 2013b), recent modelling studies have indicated that the contribution of microplastic to HOC bioaccumulation is low and overshadowed by other, natural sources (Koelmans 2015, Koelmans *et al*. 2016). Our findings support this line of reasoning, because no correlations between ingested microplastic and tissue contaminant concentrations were found (Fig. 2). Moreover, poor (if any) correlation would be expected considering the low amount, and short residence time (Grigorakis *et al*. 2017) of internalized plastics, in combination with the slow desorption kinetics of many HOCs. These observations challenge the view that microplastics can be an important source of HOCs for aquatic organisms. Interestingly, the same lack of correlation between the amount of ingested plastic and HOC concentrations was observed in northern fulmars (*Fulmarus glacialis*) from the Norwegian coast (Herzke *et al*. 2016), even though the absolute amount of plastic in the birds' stomachs was considerably greater, and gut passage times of plastic particles were orders of magnitude higher (Ryan 2015). In the light of the accumulated knowledge on MP as vectors for contaminant transport, our results further strengthen the view that the contribution of MP to contaminant bioaccumulation is likely to be negligible.

4.3 Conclusions

The results of our study indicate that microplastic ingestion by filter-feeding fish that bear a high risk of exposure is relatively low, even in a semi-enclosed sea like the Baltic, where the probability of MP loading is high. The high level of discrepancy in plastic ingestion between our and previous studies also demonstrates the urgent need to standardize gut content analysis in plastic litter research. It is also important to account for biological factors that may affect both feeding in general and selectivity towards MP as suggested by the significant effects of the fish size and reproductive state. The lack of correlation between the ingested plastic and muscle tissue HOC concentrations challenges a wide-spread view of MP being important vectors for contaminant transfer to biota.

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