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Microplastic Intake, Its Biotic Drivers, and Hydrophobic Organic Contaminant Levels in the Baltic Herring

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It is commonly accepted that microplastic (MP) ingestion can lead to lower food intake and bioaccumulation of hydrophobic organic contaminants (HOCs) in aquatic organisms. However, causal links between MP and contaminant levels in biota are poorly understood and *in situ* data are very limited. Here, we investigated whether HOC concentrations in herring muscle tissue (*Clupea harengus membras*) are related to MP ingestion using fish caught along the West coast of the Baltic Sea. The MP occurrence exhibited a large geographic variability, with MP found in 22.3% of the fish examined, and the population average being 0.9 MP ind^{-1}. However, when only individuals containing MP were considered, the average MP burden was 3.9 MP ind^{-1}. We also found that MP burden decreased with reproductive stage of the fish but increased with its body size. To predict MP abundance in fish guts, we constructed a mass-balance model using literature data on MP in the water column and physiological rates on ingestion and gut evacuation for clupeids of a similar size. The model output was in agreement with the observed values, thus supporting the validity of the results. Contaminant concentrations in the muscle tissue varied substantially across the study area but were unrelated to the MP levels in fish, suggesting a lack of direct links between the levels of HOCs and MP ingestion. Thus, despite their ubiquity, MP are unlikely to have a measurable impact on food intake or the total body burden of hydrophobic contaminants in Baltic herring.

Keywords: microplastic, Baltic sea, herring, hydrophobic organic contaminants, marine monitoring

INTRODUCTION

Plastic debris, including microplastics (MP < 5 mm), can be ingested by aquatic animals across several trophic levels (Cole et al., 2013; Lusher et al., 2013, 2015). Due to the importance of commercial fish and shellfish species for human consumption, the ingestion and presence of MP in these animals has become a matter of concern (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2016). To address this concern and to provide a quantitative assessment of MP ingestion in various fish species, an active research is ongoing (Foekema et al., 2013; Lusher et al., 2013; Rummel et al., 2016; Beer et al., 2018; Budimir et al., 2018; Markic et al., 2019).

A commonly held paradigm states that MP ingestion can lead to decreased nutritional status (Cole et al., 2015; Ogonowski et al., 2016) and bioaccumulation of hydrophobic organic chemicals (HOCs) (Rochman et al., 2013; Wardrop et al., 2016) that sorb to the MP particles in the water and desorb in the gut lumen (Rochman et al., 2014). However, some experimental and modeling studies indicate that plastic polymers could also have a net cleaning effect acting as passive samplers while in the digestive system and thereby relieve the animals of HOCs (Gouin et al., 2011; Gerdes et al., 2019; Mohamed Nor and Koelmans, 2019). The relative importance of microplastics as vectors for contaminant transport remains unresolved, possibly also due to the lack of field data linking HOC concentrations in biota to ingested MP in ecologically relevant settings.

Here, we studied MP ingestion by Baltic Sea herring (*Clupea harengus membras* L.), a commercially exploited fish and a keystone species in the Baltic food web. Being facultative pelagic filter-feeders (Huse and Toresen, 1996), herring stand a high risk of ingesting MP along with zooplankton prey and hence accumulating MP-associated contaminants. It is also a sentinel species in the Swedish National Monitoring Program for Contaminants in Marine Biota (SNMPC) and, thus, a potential indicator species for MP monitoring in the Baltic Sea (Beer et al., 2018).

If MP ingestion indeed contributes significantly to HOC bioaccumulation in contaminated environments, then one would see a positive correlation between the amount of MP ingested over time and HOC concentrations in the animals. However, there are no reliable methods to estimate accumulated MP exposure using field samples of fish, because MP do not accumulate to any significant extent in their digestive system (Jovanović, 2017). Although gut contents reflect only a recent ingestion history, the MP burden determined by gut content analysis is commonly used as a reflection of the feeding habits and habitats of the fish. Another area of concern with respect to the interpretation of MP counts in environmental samples, including fish guts, is analytical accuracy and reliability of MP extraction, and determination (Dehaut et al., 2016). Therefore, to increase the reliability of the MP gut content data, it is important to verify whether the recorded MP body burden is within ecologically plausible rates of ingestion and gut evacuation. To compare the observed MP abundance in the fish gut with the intake that can be expected given the MP abundance in the water column, and gut evacuation that can be expected given the food intake, a mass-balance modeling approach can be used. Here, we applied such modeling using literature-derived parameters on clupeid feeding and food processing as well as ambient MP concentrations, to estimate MP burden in the herring with a body size similar to those in our collection. To further evaluate the ecological plausibility of our MP-quantification, we also investigated the relationship between several biological parameters and MP ingestion.

Once the plausibility of our MP measurements was confirmed, we tested the hypothesis that HOC concentrations in the fish muscle measured by SNMPC were unrelated to the weight-specific MP gut content. Hence, the objectives and workflow of this study were: (1) to quantify MP ingestion by Baltic herring, (2) to assess whether the measured MP burden is ecologically plausible, and (3) to establish whether the levels of HOC are related to the measured MP burden in fish.

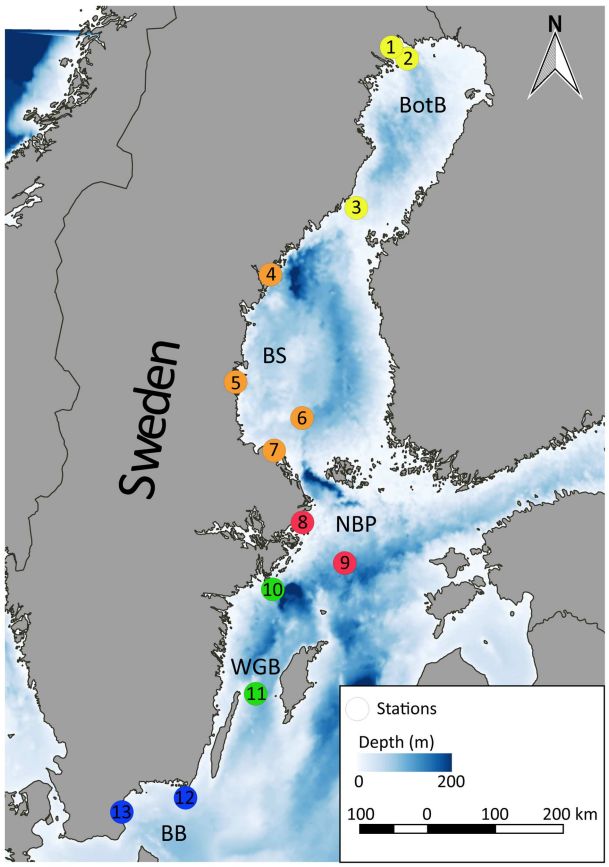

FIGURE 1 | Sampling sites within the Swedish National Monitoring Program for Contaminants in Marine Biota included in this study, BotB: Bothnian Bay (yellow), BS: Bothnian Sea (orange), NBP: Northern Baltic Proper (red), WGB: Western Gotland Basin (green), and BB: Bornholm Basin (blue). 1: Råneфjärden, 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.

MATERIALS AND METHODS

Fish Collection and Sample Characteristics

The Baltic herring used for our analyses were collected by SNMPC conducted by the Swedish Museum of Natural History (Stockholm, Sweden). To avoid possible bias by known point sources, we randomly selected 130 specimens that had been collected at 13 reference monitoring stations ($n = 10$ per station, **Figure 1**), thus covering a sufficient geographical area that would provide a representative range of HOC and MP exposure for the analysis.

The sex ratio of the selected fish was ∼50:50 and uniform across sampling sites. The individuals were 3–7 years old, with a total length of 173 ± 18 mm and body weight 35 ± 12 g

wet weight (mean ± SD). The reproductive phase determined by gametocytic maturity was classified on a five-degree scale according to Bucholtz et al. (2008) and included deformed gonads (stage 1), post spawned individuals (stage 2), juveniles (stage 3), individuals with developing gonads (stage 4), and fish with mature gonads (stage 5).

Each fish was dissected, and the muscle tissues taken from the middle dorsal muscle layer were used for HOC analysis, whereas the entire gastrointestinal tract (GIT) was used for the MP analysis. All sampling was performed according to standard procedures (TemaNord, 1995) employed by SNMPC. After dissecting, each individual GIT was packed in aluminum foil to avoid cross-contamination. All GIT samples were immediately frozen at −20°C and stored until MP analysis at the Department of Environmental Science and Analytical Chemistry, Stockholm University, Sweden.

MP Quantification in the Gastrointestinal Tract of Fish

To quantify the number of MP ingested by the herring (objective 1), each GIT was placed in a glass Petri dish, opened with surgical scissors, and rinsed with deionized, particle-free water. Using a stereo microscope, the bolus was examined, and any items resembling MP were extracted by stainless steel pincers and transferred to clean Eppendorf tubes filled with milli-Q water. The appearance of the putative MP was recorded and each particle was categorized according to its shape (fiber or fragment) and color. Hereafter, the number of MP per individual fish is referred to as *MP burden*. A subsample ($n = 20$) of the putative MP was photographed using digital camera (Canon 5D mark III) fitted to a Leica DMBR bright field microscope (Leitz, Germany). The length (fibers) or largest diameter (fragments) was measured using the segmented measurement tool in Image J (Schneider et al., 2012).

To relate HOC concentration in the muscle tissue to MP intake by the fish, the fish size must be taken into account when expressing MP counts. Moreover, in our collection, the bolus size varied considerably among the individual fish and geographical areas (**Table 2**), indicating variability in feeding activity shortly before sampling and/or spontaneous gut evacuation that might have been related to stress during the sampling. To account for variability in bolus size and the corresponding variation in the observed MP burden, we normalized individual MP counts to the gut fullness. The latter was assessed by visual observation on a five-step semi-quantitative scale: 0 (empty gut, no food items), 0.25, 0.5, 0.75, or 1 (full gut). The obtained values were further normalized to the individual body weight and termed *weight-specific MP burden* [number of MP / (gut fullness × body weight) (g wet weight)]. This normalization approach allowed for relating HOC concentrations in the fish to the expected MP burden in the GIT on a weight basis.

The following polymer identification scheme was applied. First, to identify whether the putative MP were synthetic polymers, we followed the recommendations of Hidalgo-Ruz et al. (2012). Particles 1–5 mm in diameter were recorded and classified as MP, if all the following criteria were met: (i) uniform, unnaturally bright or of an unnatural color, (ii) lack of organic structures, and (iii) uniform diameter over the entire length of a fiber. Second, to test the accuracy of the visual identification, all samples containing putative MP (i.e., the gut contents of 44 individual fish) were analyzed using Fourier transform infrared spectroscopy (FTIR) according to recent quality assurance guidelines (Hermsen et al., 2018). However, due to significant losses during initial handling, the particle recovery was low and only 61 microparticles were analyzed by FTIR. Third, MP-validation was performed by comparing the sample spectra to a published reference database (Primpke et al., 2018). The Hit Quality Index (HQI) was used to determine whether a sample spectrum matched any spectra in the database. The HQI-threshold for a match was set at 70% similarity (Thompson et al., 2004). A detailed description of the data preparation of sample spectra and sample classification is provided in **Supporting Information 1.1** and **Figure S1**.

FTIR Analysis

The infrared spectra were recorded at 4 cm^{-1} resolution with a Bruker Vertex 70 FTIR instrument that was equipped with a Bruker Platinum attenuated reflection (ATR) unit. Data were recorded on both sides of the center burst of the interferogram during forward and backward movement of the interferometer mirror. A zero filling factor of 2 was used and the spectra were apodized with a Blackman-Harris 3-term function. The spectra for samples 1–6 (**Table S1**) were recorded using an HgCdTe detector, 100 sample scans were recorded and the scanning time was 22 s. The spectra for samples 7–61 (**Table S1**) were recorded using a DTGS detector, 364 sample scans were recorded within 300 s scanning time. Individual particles were placed on the diamond crystal of the ATR unit and pressed onto the crystal with a piston. Prior to each measurement, the crystal was cleaned with 99% ethanol.

Controls and Blanks

To minimize contamination by airborne particles during the examination, the dissections were performed under a Fumex local extractor (Wesch et al., 2016); each sample being analyzed for 10 min. A Petri dish filled with filtered deionized water was placed next to a test sample to serve as a blank for the quantification and characterization of potential contamination during the analysis. When working with samples, a cotton lab coat, and gloves were used: moreover, the type and color of clothing were recorded to enable contamination back-tracing. All procedural blanks contained particles (mainly single fibers) of unknown origin. However, all these particles were <1 mm and thus did not contribute to the MP counts used in the statistical analysis. If quantifiable amounts of blank contamination with particles >1 mm were to be found, such samples would be excluded from any further analyses.

Chemical Analysis

Following the guidelines of the Swedish National Monitoring Program for Contaminants in Marine Biota, the muscle samples were analyzed for polychlorinated biphenyls (PCB 28, 52, 101, 118, 138, 153, and 180), organochlorine pesticides (DDE, DDD,

DDT, HCB, AHCH, BHCH, and Lindane), and polybrominated flame-retardants (BDE 28, 47, 99, 100, 153, 154, and HBCD). For stations 2, 7, and 9–11 (**Figure 1**), 10 g of muscle tissue from individual fish were used, whereas 1 g samples of muscle tissue from 10 individuals were pooled for samples originating from stations 1, 3–6, 8, and 12–13 (**Figure 1**). An overview of the analyzed contaminants and their average concentrations in herring muscle tissue are provided in **Table 1**, while details of the analytical procedures and quality assurance practices used by SNMPC are provided elsewhere (Bignert et al., 2016).

Data Analysis and Statistics

Relationships Between Biological Factors, Geography, and Ingested Microplastic

We used generalized additive models (GAM) in package *mgcv* to examine relationships between specific biological variables (*weight*, *gut fullness*, *age,* and *reproductive phase*) and *MP burden* (objective 2). *Sea basin* was used as a fixed factor since the inclusion of this term as a random intercept resulted in a significantly worse model (ΔAIC = 4.8). The model was specified as:

MP burden = β s(weight) + s(gut fullness) + s(age) + s(reproductive phase) + (sea basin) + ε

The multicollinearity between the explanatory variables was evaluated using concurvity measures (Amodio et al., 2014) calculated by the *mgcv*-package and found to be low (<0.24). Due to the overrepresentation of zeros in the data (overdispersion) for the MP burden, the model was constructed using zero-inflated Poisson error structure. Model performance was assessed using a quantile-quantile plot adapted for discrete data (Augustin et al., 2012) implemented in the package *mgcViz* (**Figure S5**). Differences in the MP burden between the basins were tested using Permanova with *station* nested within *basin* as a random factor (Anderson, 2001). The significance level was set at $\alpha = 0.05$; all statistical analyses were conducted in R 3.5.0 (R Core Team, 2014).

Relationships Between HOCs and Ingested Microplastic

Maximum-likelihood Factor Analysis with Varimax rotation (*factanal* function in base R) was used to assess the degree of association between the chemical variables and weight-specific MP burden in the GIT (objective 3). Whenever possible, the analysis was performed on an individual basis. When pooled samples were included, we used their arithmetic mean values. Prior to the analysis, Bartlett's test of sphericity was performed to confirm patterned relationship between the variables, which was statistically significant ($\chi^2_{15} = 176$, $p < 0.0001$). A scree plot was used to determine the number of factors to retain, and factor loadings >0.7 were considered statistically significant (MacCallum et al., 2001). When measured HOC values were below the limit of quantification (LOQ), they were imputed by LOQ divided by the square root of two (Succop et al., 2004). The analyzed chemical concentrations were summed and grouped into their respective contaminant groups (PCBs, PBDEs, and organochlorine pesticides).

To test the robustness of the approach, we also performed a follow up analysis using Generalized Additive Models following the general model structure described in section Relationships between biological factors, geography and ingested microplastic. However, instead of using MP burden as the response variable, we modeled each HOC group individually and included *weighted MP burden* as an additional explanatory variable. The models were run using normal error structure and the response variable was transformed to conform to normality using either Log10 (PCBs), Box-Cox (BDE, HBCD, and HCB), or square root transformation (organochlorine pesticides, DD). Model fit was evaluated using residual plots.

Modeling Plastic Ingestion by Herring

To evaluate whether the observed MP burden could be predicted using ambient MP abundance data and food processing rates (objective 2), we modeled the ingestion of MP using literature-derived parameters on food uptake, egestion, and MP abundance in the study area. The rationale is that observed MP abundance in the gut would reflect average exposure levels assuming that (1) MP concentrations are fairly homogeneous in the outer coastal areas (Gorokhova, 2015; Gewert et al., 2017), which are the main feeding grounds of herring (Flinkman et al., 1998); (2) the MP abundance in the water column, where the fish feed, is similar to that at the surface, where the data on the relevant size fraction of MP (1–5 mm) were collected; (3) MP ingestion by herring is non-selective and thus proportional to the MP abundance in the water; and (4) gut evacuation rates are non-discriminatory, i.e., MP are egested at the same rate as prey remains. Then, the MP burden (MP ind^{-1}) at any given time, t, can be written as the mass balance between the uptake and loss rates (Equation 1, **Figure S2**):

$$MP_t = MP\left(t - dt\right) + (IR - ER)\ dt, \tag{1}$$

where IR and ER are the ingestion and egestion rates (MP h^{-1}), respectively. They can be calculated as:

$$IR = CMP \times CR \tag{2}$$

and

$$ER = GER \times MP_t, \tag{3}$$

where CMP is the ambient MP concentration (number of MP L^{-1}), CR is the clearance rate (L h^{-1}; the volume of water swept clear of particles per individual and hour), and GER is the gut evacuation rate (h^{-1}).

We used literature data to parameterize the model (**Table S2**). The MP concentrations in the target size range (1–5 mm) from surface waters in the outer Stockholm archipelago (Gewert et al., 2017) were used as CMP values. CR values were estimated using reported feeding rates for North Sea herring on *Calanus finmarchicus,* a copepod of similar size as the microplastics considered here, and the main prey for herring (Varpe and Fiksen, 2010); see **Supporting Information 2.1** and **Figure S3** for the calculation of CR. As published gut evacuation rates for

TABLE 1 | Overview of the HOCs in herring muscle tissue and descriptive statistics of their concentrations ($\mu g\ g^{-1}$ lipid weight).

Chemical group	Chemical species	Abbreviation	Mean	SD	Median	Min	Max	LOQ
Polychlorinated biphenyls (PCBs)	2,4,4′-PCB	PCB 28	0.0039	0.0019	0.0033	0.0018	0.0106	0.002
	2,2′,5,5′-PCB	PCB 52	0.0067	0.0044	0.0057	0.0025	0.0199	0.002
	2,2′,4,5,5′-PCB	PCB 101	0.0222	0.0149	0.0176	0.0069	0.0713	0.002
	2,3′,4,4′,5′-PCB	PCB 118	0.0204	0.0135	0.0155	0.0062	0.0694	0.002
	2,2′,3,4,4′,5′-PCB	PCB 138	0.0591	0.0408	0.0444	0.0152	0.1896	0.002
	2,2′,4,4′,5,5′-PCB	PCB 153	0.0417	0.0280	0.0345	0.0120	0.1320	0.002
	2,2′,3,4,4′,5,5′-PCB	PCB 180	0.0181	0.0114	0.0149	0.0029	0.0551	0.002
Organochlorine pesticides	4,4′-DDT	DDT	0.0205	0.0218	0.0134	0.0037	0.0966	0.002
	4,4'-DDE	DDE	0.1055	0.0960	0.0815	0.0160	0.4130	0.002
	4,4'-DDD	DDD	0.0284	0.0333	0.0163	0.0016	0.1391	0.002
	α-1,2,3,4,5,6-Hexachlorocyclohexane	AHCH	0.0029	0.0005	0.0030	0.0018	0.0039	0.002
	β-1,2,3,4,5,6-Hexachlorocyclohexane	BHCH	0.0056	0.0027	0.0057	0.0018	0.0099	0.002
	γ-1,2,3,4,5,6-Hexachlorocyclohexane	Lindane	0.0029	0.0005	0.0030	0.0018	0.0039	0.002
Brominated flame retardants (BDEs)	2,4,4′-TriBDE	BDE 28	0.0002	0.0001	0.0002	0.0001	0.0005	0.0002
	2,2′,4,4′-TetraBDE	BDE 47	0.0051	0.0032	0.0041	0.0016	0.0160	0.0002
	2,2′,4,4′,5-PentaBDE	BDE 99	0.0012	0.0009	0.0009	0.0005	0.0044	0.0002
	2,2′,4,4′,6-PentaBDE	BDE 100	0.0012	0.0007	0.0011	0.0004	0.0035	0.0002
	2,2′,4,4′,5,5′-HexaBDE	BDE 153	0.0002	0.0002	0.0002	0.0001	0.0009	0.0002
	2,2′,4,4′,5,6′-HexaBDE	BDE 154	0.0006	0.0004	0.0005	0.0002	0.0018	0.0002
	1,2,5,6,9,10-Hexabromocyclododecane	HBCD	0.0114	0.0096	0.0088	0.0025	0.0470	0.002
Other	Hexachlorobenzene	HCB	0.0289	0.0209	0.0246	0.0114	0.1013	0.004

SD, standard deviation; LOQ, the limit of quantification.

adult herring were not available, we used experimental values reported for other clupeids of similar size, European pilchard (*Sardina pilchardus*) (Costalago and Palomera, 2014) and South American pilchard (*Sardinops sagax*) (van der Lingen, 1998), which have similar feeding ecology and physiology as Baltic herring (Collard et al., 2017). The physiological rates used in the model corresponded to the average size of our fish.

The model was implemented using STELLA® ver. 9.4.1 software (iSee systems, Inc. Lebanon, NH, U.S.A.) to estimate MP burden (MP ind^{-1}) dynamics in a fish population at a given MP abundance. The intrapopulation variability was simulated using a Monte Carlo generator with 1,000 permutations; details on the simulation settings are provided in **Supporting Information 2.2** and **Figure S4**. To validate the model, we compared the simulated data distribution from the model to the field data using descriptive statistics, χ^2, and the two-sample Cramér-von Mises tests.

RESULTS

Observed MP Burden

Particles identified by visual inspection as MP (the frequency of occurrence, %FO, **Table 2**) were found in 44 out of the 130 individuals (33.8%; range: 1–51 pieces of putative plastic fibers or fragments ind^{-1}). In these 44 individuals, the mean abundance was 7.8 ± 12.2 particles ind^{-1} (± SD) and the average length of the MP was 2.39 ± 1.80 mm; width was 0.022 ± 0.10 mm (± SD, all measured MP were fibers). The dominant type of particles were fibers of various colors (87.6%), while fragments were less frequent (12.4%). However, only 32.8% of the visually identified, putative MP were classified as either synthetic or semi-synthetic (viscose) by FTIR and 47.5% were classified as being of natural origin (e.g., chitin, fur, and cellulose); 19.7% could not be identified (**Table S1**).

After correcting for the proportion of misclassified samples, only 29 individuals containing MP were retained (22%; range: 1 to 17 MP), with a mean abundance of 3.9 MP ind^{-1} ± 4.4 SD. When all examined individuals were considered, the population average was 0.9 MP ind^{-1} ± 2.6 SD, with the 95% bootstrap confidence interval ranging 0.5–1.4 MP ind^{-1}. The variation in the MP burden between the stations and basins was high (**Figure 2**; **Table 2**) and no significant differences in the MP burden between the basins were found (*station* nested within *basin* as a random factor, pseudo $F_{(4,117)} = 0.9$, $p = 0.48$).

Predicted vs. Observed MP Burden and Frequency of Occurrence

The model predicted that 81% of fish contained MP, with a mean MP burden of 4.7 MP ind^{-1}; these values were about 5-fold the observed values. However, the ranges of the frequency distributions for the simulated and observed values were overlapping, although the field observations were more strongly skewed toward zero values compared to the model prediction (**Figure 3A**; **Table S3**). The difference between the distributions was statistically significant (Cramér-von Mises $T = 226$, $p < 0.0001$). However, when zero values were excluded, the distributions, albeit still significantly different (Cramér-von

TABLE 2 | Descriptive statistics for the microplastics recovered from the gastrointestinal tract of Baltic Sea herring as well as gut fullness of the examined fish.

Sea basin	Fibers (min-max)				Fragments (min-max)			MP total				GF	
	Black/Brown	Red	Blue	Clear	Black/Brown	Red	Blue	FO (%)	Median	Mean all samples	Mean of presences	Range (min-max)	% fish with empty GIT	Median	IQR
Bothnian bay	0–7	0–38	0–0	0–3	0–0	0–3	0–12	46.7 (40.0)	0	4.1 (1.4)	8.9 (3.9)	0–40 (0–15)	10	50	25–50
Bothnian sea	0–8	0–18	0–0	0–5	0–7	0–0	0–2	30.0 (12.5)	0	1.3 (0.4)	4.2 (3.0)	0–18 (0–6)	7.5	25	25–50
Northern baltic proper	0–1	0–0	0–0	0–51	0–0	0–2	0–2	30.0 (20.0)	0	6.7 (2.15)	22.2 (10.8)	0–51 (0–17)	25	25	19–50
Western gotland basin	0–1	0–3	0–1	0–1	0–0	0–7	0–0	40.0 (25.0)	0	1.0 (0.3)	2.5 (1.2)	0–7 (0–2)	10	25	25–25
Bornholm basin	0–1	0–13	0–0	0–2	0–0	0–0	0–0	20.0 (15.0)	0	0.9 (0.3)	4.5 (2.0)	0–13 (0–4)	0	50	25–56
Baltic sea (all basins pooled)	0–8	0–38	0–1	0–51	0–7	0–7	0–12	33.8 (22.3)	0	2.7 (0.9)	7.8 (3.9)	0–51 (0–17)	9.2	25	25–50

For each basin, the number of differently colored fragments and fibers that were recovered from the fish are shown as well as MP frequency of occurrence (FO), median, range (min-max), and mean; moreover, the mean values were calculated for all fish including zero observations and for the fish that only contained putative MP in their GIT (i.e., excluding zero values). Gut fullness (GF) is presented as percent of fish with empty stomachs, median gut fullness, and the corresponding inter-quartile range (IQR). Values in parentheses represent FTIR-validated MP data. Data are ordered north to south. All values in this table are based on untransformed counts whereas corresponding data in ***Figure 2*** *is log10-transformed.*

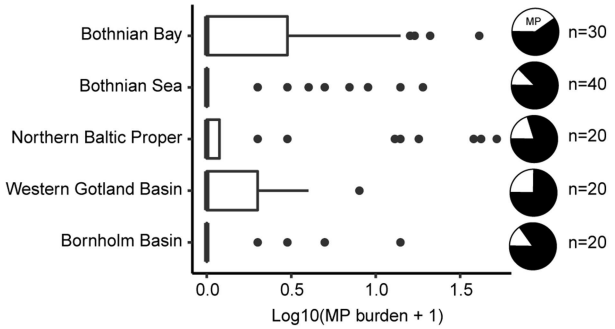

FIGURE 2 | Boxplot of Log_{10}-transformed MP abundance in the gastrointestinal tract (GIT) of herring per basin; the basins are ordered from north to south. Data are presented as medians (vertical lines), inter quartile range, IQR (boxes), 1.5 IQR (whiskers) and outliers (points) deviating > 1.5 IQR. The black slices of the pie charts indicate the proportion of the fish with no MP in the GIT. Corresponding, untransformed data is provided in **Table 2**.

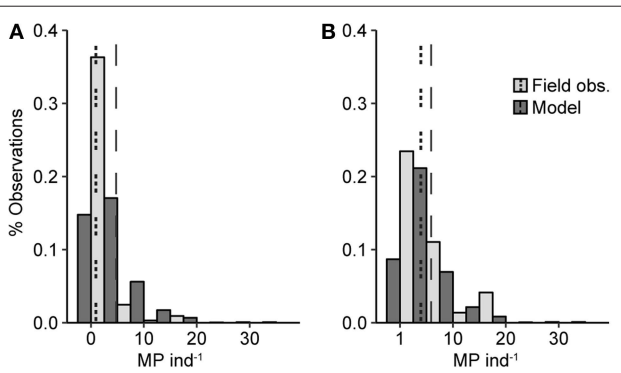

FIGURE 3 | Frequency distribution of the MP burden based on the model simulations (dark gray bars) and field observations (light gray bars). **(A)** shows the entire dataset and **(B)** presents only fish with MP in the GIT (i.e., the non-zero values). The dashed vertical lines indicate the mean values for the model simulations (long dash) and the observations (short dash).

Mises $T = 12.5$, $p < 0.01$), became more similar (**Figure 3B**; **Table S3**), indicating that much of the difference between the distributions was driven by the significantly higher proportion of zero observations in the field data ($\chi^2 = 219.5$, $p < 0.0001$).

Linkage Between MP Intake and HOCs

We found no relationship between the weight-specific MP burden and the concentration of any of the HOCs (**Figure 4**). Together, the two factors explained a cumulative variance of 84.3% (**Table S5**). As a variable, weight-specific MP burden loaded weakly and negatively (−0.14) on the first axis and moderately positive (0.58) on the second axis. In contrast, the organochlorine pesticides and PBDEs loaded significantly and positively on the first axis, while the PCBs loaded moderately positive (0.56) on the first and significantly positive (0.82) on the second axis. Hence, no contaminant group had loadings clustering with those for the weight-specific MP burden. The follow-up GAM analysis confirmed the lack of any significant

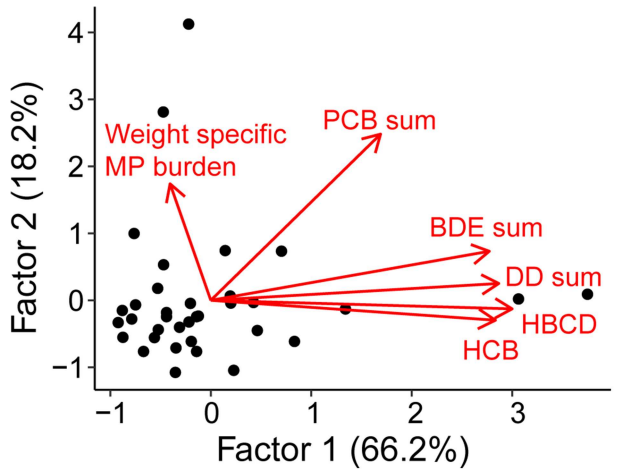

FIGURE 4 | Factor scores (axes) and loadings (arrows) of contaminants (HBCD, HCB and the sum of PCBs, BDEs, and DDs) and weight-specific MP burden. The data included in this analysis were weighted MP burden and HOC concentrations in herring muscle tissue determined in (1) individual fish specimens, and (2) pooled fish samples (10 ind. per sample).

relations between MP and HOC groups (PCBs, BDEs, DDs, HBCD, and HCB, **Table S6** and **Figure S6**).

Biological Factors Related to MP Burden

The GAM explained 64.7% of the deviance (**Table S4**) and indicated that the MP burden was positively and nearly linearly related to fish *body weight* (GAM, $\chi^2 = 16.8$, $p < 0.001$, **Figure 5A**). In contrast, a negative effect was found for *reproductive phase*, where MP burden was significantly lower in fish that had reached sexual maturity (GAM $\chi^2 = 11.1$, $p < 0.05$, **Figure 5B**). *Gut fullness* only had a negative effect on MP burden when the GIT was empty of food items (GAM $\chi^2 = 41.3$, $p < 0.0001$, **Figure 5C**), while *Age* displayed a negative relationships with MP burden (GAM $\chi^2 = 18.8$, $p < 0.0001$, **Figure 5D**).

DISCUSSION

Microplastics Are Common but Not Abundant in Herring Guts

Microplastics (mostly fibers of synthetic and semi-synthetic origin) were found in about 20% of the fish. While these values are in good agreement with those reported for herring by Beer et al. (2018) for the central Baltic Sea (i.e., 20% containing MP, with 93% fibers), other studies report considerably lower MP frequency of occurrence and fiber contribution to total MP in herring. Both Foekema et al. (2013) and Rummel et al. (2016) found plastics in only 2% of herring samples from the North Sea and the Southern Baltic Sea, with fibers accounting for <10% of MP. Excluding fibers from their analyses, Budimir et al. (2018) reported a frequency of occurrence as low as 1.8% in herring from the northern Baltic Sea. Our results suggest that these discrepancies between different studies could be related to differences in fish size, gut fullness and ontogenetic diet shifts. For example, Foekema et al. (2013) used fish that were considerably larger (>200 mm total length) and most likely already had switched from filter feeding to raptorial feeding on larger prey (Huse and Toresen, 1996). This change in feeding mode would result in a lower ingestion rate of zooplankton-sized plastic particles and thus in a lower overall MP burden. In the study of Rummel et al. (2016), many fish stomachs were empty, which probably was related to arrested feeding in concert with spawning (Stacey and Hourston, 1982), and possibly, stress-induced gut evacuation caused by the fish sampling (Wilkins, 1967; Vinson and Angradi, 2011). In addition, one would expect the amount of ingested MP to scale with the absolute size of bolus or gut fullness. However, since this relationship was weak (**Figure 5C**), our findings only partly support this expectation. One possible explanation for this could be a slower egestion of MP compared to prey, similar to the selective retention of plastic fibers in amphipods (Au et al., 2015) and fragments in cladocerans (Ogonowski et al., 2016). The slower egestion would result in a temporary accumulation of MP in the fish gut and obscure the expected positive relationship between the gut fullness and MP burden. While fish weight appears to be the strongest co-variable for standardizing gut MP content, gut fullness was also influential, particularly for fish with empty guts, which may occur during fasting periods that are normally observed during spawning time (Stacey and Hourston, 1982). The latter was further supported by the negative effect of *reproductive phase* on MP burden. Although the effect of *Age* also was statistically significant, the effect was not particularly strong and most probably of lesser biological importance.

The range of the MP burden predicted by our simple model was similar to that observed in the field-caught specimens, although the proportion of fish predicted to contain MP was more than 5-fold higher (**Figure 3A**). This is, however, not surprising because, the frequency of zero values was driven by the variability in MP occurrence in the water that was derived from surface-collected MP. Moreover, the model assumed homogeneous MP distribution in the water column, which is unlikely, because the MP distribution is patchy, varying with depth (Gorokhova, 2015). In addition, MP can form aggregates that are too large to be mistaken for zooplankton prey (Lagarde et al., 2016). Therefore, the distribution of MP concentrations originating from the surface collections and used to model MP encounter rate might poorly reflect the actual abundance of MP available to the fish. The observed MP burden for the population was also more variable, which is likely to be related to diel variations in feeding and gut evacuation under natural conditions (Seyhan and Grove, 2003), not accounted for by the model. Other biological factors, such as maturity level, ontogenetic changes in feeding, and behavior, may have affected the probability of MP ingestion and thus contributed to the intrapopulation variability in the MP burden. Finally, fishing methods (which may induce spontaneous gut evacuation) and time of capture (which may reflect diurnal differences in feeding activity) may have contributed to the observed discrepancy in the MP burden distribution. Nevertheless, given the simplicity of the model and the uncertainties associated with its parameters, the predicted values were sufficiently close to those found in the field, indicating that MP uptake can be predicted provided that we have reliable MP abundance estimates.

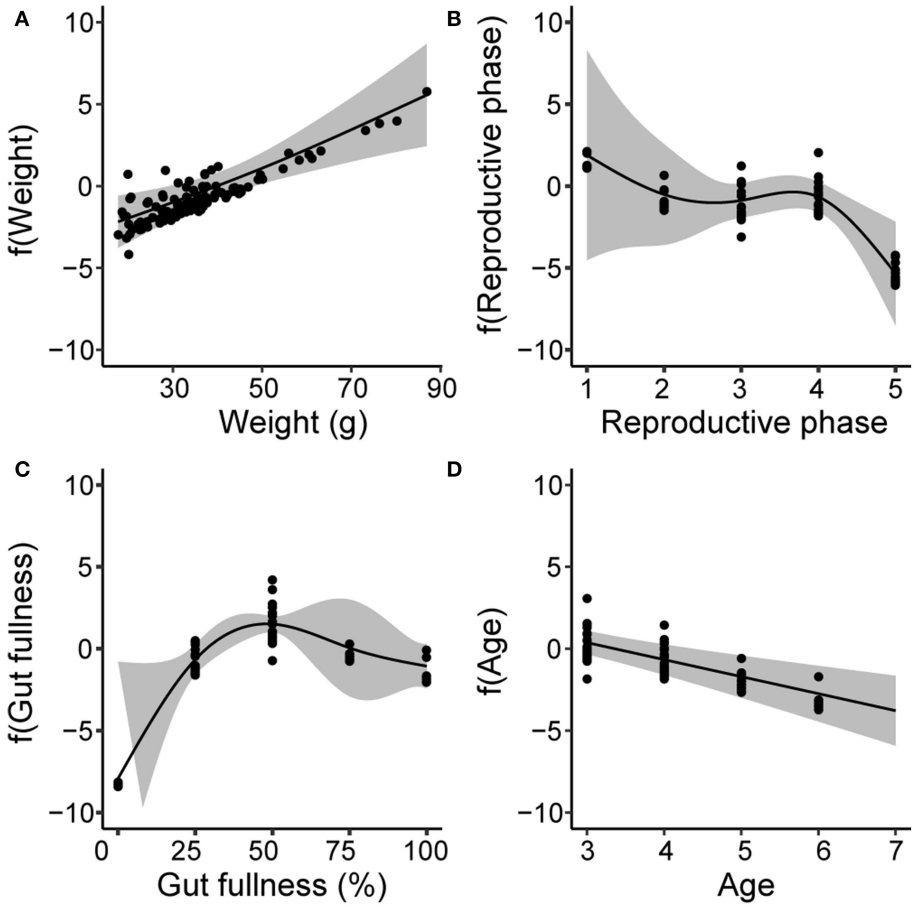

FIGURE 5 | Generalized additive models (GAMs) showing partial response curves for the explanatory biological variables: *body weight* **(A)**, *reproductive phase* **(B)**, *gut fullness* **(C)** and *age* **(D)**. The classes for *reproductive phase* correspond to: 1 = deformed gonads, 2 = post spawned, 3 = juvenile, 4 = developing gonads, and 5 = mature gonads. The vertical axis shows the relative influence of the explanatory variable on the prediction of MP burden on the base of partial residuals. Gray bands indicate 95% confidence interval for each curve.

No Correlation Between Weight-Specific MP Burden and HOCs

The transfer of hydrophobic contaminants from ingested plastics to biota has been described as the so-called "Trojan horse" effect (Cole et al., 2011). While this transfer has been demonstrated under laboratory conditions (Besseling et al., 2013; Batel et al., 2016), recent modeling studies indicate that natural sources are much more important than MP in explaining HOC bioaccumulation patterns in aquatic organisms (Mohamed Nor and Koelmans, 2019). We did not find any correlation between HOC concentrations in herring muscle and MP burden. It could be argued, however, that omitting small MP (<1 mm) from our analysis, could have biased the results. Indeed, by focusing on the larger MP, we ignored the potentially important influence of a higher total surface area and thus higher HOC desorption rates (Hendriks et al., 2001; Hartmann et al., 2017) of smaller plastic fragments. From the fish feeding biology point of view, however, the ingestion of such small particles by fish of this size is rather unlikely, because filter-feeding herring have a relatively low capacity to retain small particles due to their rather wide gill raker spacing (Collard et al., 2017) and actively avoid smaller prey by raptorial feeding (Aro et al., 1989). This line of reasoning has also been supported by several other studies reporting a predominant retention of larger than 1-mm MP by herring of similar size as in our study (Lenz et al., 2016; Collard et al., 2017; Beer et al., 2018). In the European pilchard (*Sardina pilchardus*), Digka et al. (2018) analyzed the presence of anthropogenic microparticles (1.2–5,000 μm) in the fishes' gastrointestinal tract using hydrogen peroxide digestion and found ∼75% of the particles to be in the size range of the natural food, further supporting primary ingestion as the main route for microplastic uptake in clupeids. Moreover, given the short residence time (Grigorakis et al., 2017) of ingested particles and the slow desorption kinetics of many HOCs, the lack of correlation between the MP and organic contaminants is rather expected and in line with other reports for fish and other aquatic animals (Herzke et al., 2016; Kleinteich et al., 2018; Gerdes et al., 2019).

Causality is difficult to prove using environmental samples, where many different parameters concurrently

affect contaminant body burden of an organism (Hartmann et al., 2017), including various biotic factors that have significant effects on both MP (this study) and HOC levels (Persson et al., 2013). However, our findings suggest that there is no tenable relationship between the MP intake and tissue contaminant concentrations in the Baltic herring (**Figure 4**). Similarly, no correlation has been found between the amount of ingested plastic and HOC concentrations in northern fulmars (*Fulmarus glacialis*) from the Norwegian coast (Herzke et al., 2016), even though the birds had ingested much larger amounts of plastic and their gut passage time for plastic debris is several orders of magnitude longer than in herring (Ryan, 2015). This lack of relationship is also supported by the relatively constant MP burden observed in Baltic herring over the past three decades (Beer et al., 2018), while muscle concentrations of HOCs have decreased significantly (Bignert et al., 2016). The mass-balance model indicates that our measurements of the MP burden are ecologically plausible given the currently reported abundances of MP in the Baltic surface water, thus supporting the reliability of the MP burden estimates in the Baltic herring reported here and in other studies and providing confidence in the methods employed. Taken together, these findings contrast the currently held paradigm that microplastics are an important source and vector of HOCs for aquatic organisms (Mato et al., 2001; Rochman et al., 2013).

CONCLUSIONS

Our findings suggest that in a semi-enclosed sea like the Baltic, where the MP loading is expectedly high, the frequency of occurrence of microplastic in planktivorous fish is moderate. In agreement with other studies, we also found that biological factors, such as fish size and the reproductive state may affect both feeding in general and selectivity toward MP, and, hence, the MP burden. However, we found no indication that HOC concentrations in the muscle tissue are related to the amount of ingested plastic in the fish. Thus, our study further strengthens the view that MP contribute extremely little to the herring diet and play a negligible role in explaining contaminant bioaccumulation in the fish.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The fish caught for this study were collected under license N83/14 and complied with the standards and procedures stipulated by the Swedish Ministry of Agriculture.

AUTHOR CONTRIBUTIONS

EG conceived the study. MO and EG conducted the data analysis and were mainly responsible for manuscript writing. VW performed the microplastic extraction from fish guts and performed the primary visual microplastic identification. SD coordinated the fish sampling, provided the gastrointestinal tracts from herring and was responsible the HOC chemical analyses. AB and EH-B performed the FTIR-analyses and contributed to manuscript writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2019.00134/full#supplementary-material

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