**Supporting information for**

**Microplastic intake, its biotic drivers, and hydrophobic organic contaminant levels in the Baltic herring**

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1.1 Data handling of FTIR spectra

FTIR spectra were corrected for atmospheric CO2 absorption by subtraction of a CO2 spectrum and baseline corrected according to Primpke et al. (2018). To reduce noise and enhance the spectrum quality without losing subtle spectral information, each spectrum passed through a baseline correction and denoising procedure using a second order derivative Savitzky-Golay filter (Renner et al. 2019) as implemented in Unscrambler® X (version 10.5.1; Camo Software, 2018). Both pre-processed and original spectra were then compared to the spectral libraries assembled by Primpke and co-workers (Primpke et al. 2018) using BioRad KnowItAll® Informatics System software. The Correlation algorithm implemented in the software was used to evaluate each query spectrum to the spectra of the databases. The most appropriate match (Hit Quality Index; HQI) was selected based on matching peak wavenumber positions. The scale 0 – 100% was used, with 100 being the best possible match, and a minimum 70% HQI between unknown and matched spectra was accepted as a threshold. Moreover, in a ranked list of the database hits, the difference, or gap in the HQI between two successive hits was used as an indicator of the match quality. When several hits were followed by a 10% gap, all these hits were treated as equally plausible matches[[1]](#footnote-1). In all spectra, we focused on the 3799 to 850 wavenumber (cm-1) region, excluding the interval 2690 to 1900 wavenumber (cm-1) which corresponds to the absorption range of the diamond ATR crystal.

* + 1. *Microparticle classification*

The putative microplastic particles found in the gastrointestinal tract of herring by visual identification were classified as either *microplastic*, *semi-synthetic* or *natural* based on the combination of visual information and FTIR spectra (Figure S1, Table S1). When all FTIR spectra showed a closest match with synthethic materials, the particle was classified as *synthethic*. If some spectra matched natural material but the particle color was of unnatural (bright red or blue) the particle was classified as *semi-synthethic*. Particles with natural color and at least one match with a natural material were classified as *natural*.

2.1 Model parameterization

*2.1.1 Clearance rate (CR)*

Clearance rates (CR) for Baltic herring were calculated based on intake rates of *Calanus finmarchicus* in the North Sea (Fig. 5 in Varpe and Fiksen 2010), which is the main prey for herring in this area. These copepods are also of similar size (2-3 mm prosome length) as the microplastic particles considered in this study (Pasternak et al. 2004). We used the reported values on *C. finmarchicus* consumption by herring expressed as (J copepod [J herring]-1 day-1) and ambient *C. finmarchicus* abundance to obtain the CR.

The average CR of the Baltic herring population examined in this study (L h-1) was calculated assuming an energy content of 3.5 kJ and 10 kJ g wet weight-1 for *C. finmarchicus* and herring, respectively (Varpe et al. 2005). The average weight of the sampled Baltic herring (35 g) was used to derive the consumption rate on an individual basis using a first-order exponential decay function fitted to data on the CR and prey abundance for the North Sea herring feeding on *C. finmarchicus* (Figure S3). The asymptote value (1.04 × 103 L ind.-1 h-1) was assumed to represent CR of the Baltic herring, because mesozooplankton abundance in the Baltic Sea normally supersede the maximum reported abundance for *C. finmarchicus* in the North Sea (Varpe and Fiksen 2010, Gorokhova et al. 2016).

*2.1.2 Ambient MP concentrations in the Baltic Sea (CMP)*

We used the average microplastic concentrations reported by Gewert et al. (2017) in the outer Stockholm archipelago (0.58 MP m-3) estimated by surface manta trawls (335 µm mesh). These values were used, because the size range (median MP size and inter quartile range, IQR: fragment diameter = 1 mm (IQR 0.6-1.5 mm), fiber length = 1 mm (IQR 1-3 mm)) fits well the size of MP recovered from the fish guts. Also, the polymer materials have been rigorously identified by FTIR in this selection of the field-collected MP, thus ensuring that the fragments collected were indeed microplastics.

*2.1.3. Gut evacuation rates (GER)*

We were not able to find data on gut evacuation rates for adult herring; therefore, a lower and an upper limit reported for two clupeid species of similar size and feeding ecology as the herring analyzed here (Collard et al. 2015) were chosen. The lower limit (0.05 h-1) was adopted from the experimental and field data collected for adult South American pilchard (*Sardinops sagax*) (van der Lingen 1998). The upper limit (0.26 h-1) was experimentally derived for adult European pilchard (*Sardina pilchardus*) (Costalago and Palomera 2014).

**2.2 Monte Carlo simulation of MP burden in the Baltic herring**

To estimate MP burden (MP ind-1) dynamics at a given MP abundance from time 0 to the point when it is stabilized (48 h), we performed Monte Carlo simulation with 1000 permutations using STELLA® ver. 9.4.1 software (iSee systems, Inc. Lebanon, NH, U.S.A.), with the equations (Eqs. 1 to 3) integrated as shown in Figure S4. Ambient MP concentrations (CMP) were allowed to vary randomly following a Poisson distribution as were the data presented in Gewert et al. (2017), whereas the CR values were normally distributed with a mean and SD of 1041 L h-1 and 27 h-1, respectively, and GER values varied randomly between 0.05 and 0.26 h-1 without any assumption regarding the distribution (Table S2). The final value of each run was used to represent an individual in the population.

SI Tables and Figures

Table S1. FTIR classification results based on the comparison with a reference database (Primpke et al. 2018). A match to one or more equally plausible compounds was accepted at HQI ≥ 70%. Spectra with HQI-scores below this threshold were classified as unknown.

| Sample | Sample type | FTIR Classification | Group |
| --- | --- | --- | --- |
| 1 | transparent fiber | fur | natural |
| 2 | transparent fiber | polyvinyl | synthetic |
| 3 | blue fiber | plant fiber, cellulose, hydroxypropyl methyl cellulose, polyvinyl alcohol | semi-synthetic |
| 4 | transparent fiber | algae | natural |
| 5 | transparent fiber | fur | natural |
| 6 | transparent fiber | algae | natural |
| 7 | red fiber | fur | natural |
| 8 | black fiber | plant fiber | natural |
| 9 | black fiber | plant fiber, cellulose | natural |
| 10 | blue fiber | plant fiber, viscose | semi-synthetic |
| 11 | blue fiber | viscose, polyvinyl | semi-synthetic |
| 12 | transparent fiber | chitin | natural |
| 13 | blue fiber | unknown | unknown |
| 14 | blue fiber | unknown | unknown |
| 15 | blue fiber | plant fiber, cellulose, hydroxypropyl methyl cellulose, polyvinyl alcohol | semi-synthetic |
| 16 | red fiber | plant fiber, cellulose, hydroxypropyl methyl cellulose, rubber | semi-synthetic |
| 17 | black fiber | unknown | unknown |
| 18 | white fragment | acrylonitrile butadiene styrene, alkyd varnish | synthetic |
| 19 | blue fiber | unknown | unknown |
| 20 | black fiber | unknown | unknown |
| 21 | red fiber | rubber, ethylene propylene, poly 1-butene isotactic | synthetic |
| 22 | brown fragment | polyamide, fur | synthetic |
| 23 | black fiber | rubber, poly 1-butene isotactic | synthetic |
| 24 | black fiber | rubber, polyethylene | synthetic |
| 25 | blue fiber | fur | natural |

Table S1 continued

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Sample type | FTIR Classification | Group |
| 26 | red fiber | unknown | unknown |
| 27 | red fiber | fur, zein | natural |
| 28 | red fiber | fur, zein | natural |
| 29 | red fiber | fur, zein | natural |
| 30 | red fiber | fur, zein | natural |
| 31 | red fiber | fur, zein | natural |
| 32 | red fiber | fur, zein | natural |
| 33 | red fiber | plant fiber, wood, hydroxyethyl cellulose | semi-synthetic |
| 34 | blue fiber | plant fiber, viscose, cellulose, wood, hydroxyethyl cellulose | semi-synthetic |
| 35 | black fiber | unknown | unknown |
| 36 | blue fiber | unknown | unknown |
| 37 | white fiber | fur, animal fiber, zein | natural |
| 38 | black fiber | hydroxypropyl methyl cellulose, ethyl cellulose, hydroxypropyl cellulose | synthetic |
| 39 | white fragment | fur, chitin | natural |
| 40 | black fiber | plant fiber, hydroxypropyl methyl cellulose, viscose | semi-synthetic |
| 41 | black fiber | cellulose, plant fiber, viscose, wood | natural |
| 42 | black fiber | plant fiber, cellulose, hydroxyethyl cellulose | natural |
| 43 | black fiber | hydroxyethyl cellulose, wood, plant fiber, cellulose | natural |
| 44 | black fiber | cellulose, plant fiber, wood, hydroxyethyl cellulose | natural |
| 45 | black fragment | algae, plant fiber, viscose, wood, cellulose | natural |
| 46 | black fragment | polyurethane acrylic resin, alkyd varnish | synthetic |
| 47 | brown fiber | plant fiber, methyl cellulose | natural |
| 48 | brown fragment | polyethylene, ethylene propylene, rubber | synthetic |
| 49 | brown fragment | honeycomb, plant fiber, algae | natural |
| 50 | black fiber | polyester, polyethylene terephthalate | synthetic |
| 51 | brown fiber | unknown | unknown |
| 52 | black fiber | viscose, plant fiber | natural |
| 53 | black fragment | algae, plant fiber | natural |
| 54 | red fiber | algae | natural |
| 55 | black fiber | unknown | unknown |
| 56 | red fiber | chitin, fur | natural |

Table S1 continued

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Sample type | FTIR Classification | Group |
| 57 | black fiber | plant fiber, wood, viscose | semi-synthetic |
| 58 | blue fiber | fur, animal fiber | natural |
| 59 | black fiber | plant fiber, wood, viscose | semi-synthetic |
| 60 | black fiber | unknown | unknown |
| 61 | black fiber | unknown | unknown |

Table S2. Variables and simulation settings used to model microplastic ingestion in Baltic Sea herring. Details regarding derivation of the values are provided in the Supporting Information 2.1.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Parameter | Unit | Average | Min | Max | S.D | Distribution | Species | Meaning | Reference |
| CMP | MP L-1 | 5.8 × 10-4 |  |  |  | Poisson |  | MP concentration in the water column | Gewert et al. 2017 |
| CR | L ind.-1 h-1 | 1.04 × 103 |  |  | 2.6 × 102 | Normal | *Clupea harengus* | Clearance rate | Varpe & Fiksen 2010 |
| GER1 | h-1 |  | 5 × 10-2 | 2.6 × 10-1 |  |  | *Sardinops sagax, Sardina pilchardus* | Gut evacuation rate | Van der Lingen 1998, Costalago & Palomera 2014 |
| IR | MP h-1 |  |  |  |  |  |  | Number of MP ingested at time *t* |  |
| MP | MP |  |  |  |  |  |  | Number of MP in fish stomach at time *t* |  |
| ER | MP h-1 |   |   |   |   |   |   | Number of MP egested at time *t* |   |

The lower value for GER is based on data for *Sardinops sagax* (Van der Lingen 1998) while the higher is derived from *Sardina pilchardus* (Costalago & Palomera 2014).

Table S3. Descriptive statistics for the predicted (modelled) and observed distributions of the MP burden in the Baltic herring. The data are presented as either “Total”, i.e., where individuals without MP in the GIT are included, or “Zeros excluded” that shows only the fish with positive MP burden.

|  |  |  |
| --- | --- | --- |
|   | **Total** | **Zeros excluded** |
|   | Mod | Obs | Mod | Obs |
| n | 1000 | 130 | 806 | 29 |
| Mean | 4.7 | 0.9 | 5.9 | 3.9 |
| SD | 4.7 | 2.6 | 4.5 | 4.4 |
| Median | 3.6 | 0 | 4.4 | 2 |
| Min | 0 | 0 | 1.3 | 1 |
| Max | 33.3 | 17 | 33.3 | 17 |
| Range | 33.3 | 17 | 32 | 16 |
| Skew | 1.8 | 4.2 | 2 | 1.6 |
| Kurtosis | 5 | 18.2 | 5.7 | 1.4 |
| SE | 0.1 | 0.2 | 0.2 | 0.8 |

**Table S4.** Summary statisticsfor the generalized additive model showing the degrees of freedom for the smoothing parameter (edf), χ2-statistic, model estimate (Est.), standard error (S.E.), z-statistic and p-value.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variable** | **edf** | **χ2** | **Est.** | **S.E.** | **z** | **p** |
| s(Weight) | 1.44 | 16.8 |  |  |  | 0.0002 |
| s(Reproductive phase) | 3.26 | 11.1 |  |  |  | 0.02 |
| s(Gut fullness) | 2.86 | 41.3 |  |  |  | <0.0001 |
| s(Age) | 1.00 | 18.8 |  |  |  | <0.0001 |
|  |  |  |  |  |  |  |
| (Intercept) |  |  | -8.9 | 3.0 | -3.0 | 0.003 |
| Western Gotland Basin |  |  | 7.3 | 2.9 | 2.6 | 0.01 |
| Northern Baltic proper |  |  | 9.9 | 3.4 | 2.9 | 0.003 |
| Bothnian Sea |  |  | 6.8 | 3.3 | 2.1 | 0.04 |
| Bothnian Bay |  |  | 7.3 | 3.3 | 2.2 | 0.03 |
|  |  |  |  |  |  |  |
| **Deviance explained** |  |  |   |   |   | 64.7% |

**Table S5.** Summary statistics and factor loadings for the variables used in the factor analysis. WS MP burden = weight specific MP burden. Factor loadings > 0.7 are considered statistically significant.

|  |  |  |
| --- | --- | --- |
|   | **Factor 1** | **Factor 2** |
| WS MP burden | -0.135 | 0.578 |
| BDE sum | **0.921** | 0.243 |
| HBCD | **0.997** | -0.042 |
| DD sum | **0.953** | 0.083 |
| HCB | **0.941** | -0.099 |
| PCB sum | 0.562 | **0.824** |
|  |  |  |
| SS | 3.969 | 1.091 |
| Proportion var | 0.662 | 0.182 |
| Cumulative var | 0.662 | 0.843 |

**Table S6.** Summary statisticsfor the confirmatory generalized additive models showing the degrees of freedom for the smoothing or fixed parameter (edf, df), F-statistic, p-value and deviance explained (dev. (%)) per modeled HOC-group. The initial model included all terms while the final model included only significant terms after backward variable selection (sequential removal of insignificant terms). Residual plots for the initial model are provided in Figure S6.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  | **Initial model** |  **Final model** |  |
| **Model** | **Dependent** **variable** | **Explanatory** **variable** | **(e)df** | **F** | **p** | **dev. (%)** | **(e)df** | **F** | **p** | **dev. (%)** |
| 1 | PCBs | s(Weight) | 1.91 | 0.81 | 0.41 | 67.4 |  |  |  | 47.9 |
|  |  | s(Reproductive phase) | 1.00 | 0.001 | 0.98 |  |  |  |  |  |
|  |  | s(Age) | 1.16 | 0.40 | 0.53 |  |  |  |  |  |
|  |  | s(Weighted MP burden) | 1.65 | 1.59 | 0.23 |  |  |  |  |  |
|  |  | s(Gut fullness) | 2.06 | 1.26 | 0.24 |  |  |  |  |  |
|  |  | Basin | 4.00 | 3.18 | 0.03 |  | 4.00 | 6.90 | <0.001 |  |
| 2 | BDEs | s(Weight) | 1.78 | 0.62 | 0.51 | 76.5 |  |  |  | 53.6 |
|  |  | s(Reproductive phase) | 1.00 | 4.03 | 0.06 |  | 1.00 | 24.30 | <0.001 |  |
|  |  | s(Age) | 2.59 | 2.00 | 0.14 |  |  |  |  |  |
|  |  | s(Weighted MP burden) | 1.00 | 1.51 | 0.23 |  |  |  |  |  |
|  |  | s(Gut fullness) | 2.39 | 2.88 | 0.05 |  | 2.33 | 3.57 | 0.04 |  |
|  |  | Basin | 4.00 | 2.81 | 0.05 |  |  |  |  |  |
| 3 | DDs | s(Weight) | 3.24 | 9.93 | <0.001 | 96.0 | 3.28 | 14.54 | <0.001 | 95.6 |
|  |  | s(Reproductive phase) | 1.00 | 0.99 | 0.33 |  |  |  |  |  |
|  |  | s(Age) | 1.00 | 8.43 | 0.01 |  | 1.00 | 14.10 | <0.001 |  |
|  |  | s(Weighted MP burden) | 1.00 | 0.16 | 0.69 |  |  |  |  |  |
|  |  | s(Gut fullness) | 1.90 | 3.47 | 0.05 |  | 1.63 | 4.64 | 0.02 |  |
|  |  | Basin | 4.00 | 18.80 | <0.001 |  | 4.00 | 33.42 | <0.001 |  |
| 4 | HBCD | s(Weight) | 1.52 | 0.23 | 0.73 | 82.8 |  |  |  | 77.4 |
|  |  | s(Reproductive phase) | 1.00 | 8.49 | 0.01 |  | 1.00 | 10.20 | <0.01 |  |
|  |  | s(Age) | 1.00 | 6.14 | 0.02 |  | 1.00 | 7.57 | 0.01 |  |
|  |  | s(Weighted MP burden) | 1.00 | 0.10 | 0.76 |  |  |  |  |  |
|  |  | s(Gut fullness) | 1.93 | 2.07 | 0.16 |  |  |  |  |  |
|  |  | Basin | 4.00 | 1.79 | 0.17 |  | 4.00 | 2.58 | 0.06 |  |
| 5 | HCB | s(Weight) | 1.00 | 0.02 | 0.88 | 76.2 |  |  |  | 73.0 |
|  |  | s(Reproductive phase) | 1.00 | 5.64 | 0.03 |  | 1.00 | 5.86 | 0.02 |  |
|  |  | s(Age) | 1.20 | 4.29 | 0.06 |  | 1.38 | 5.54 | 0.03 |  |
|  |  | s(Weighted MP burden) | 1.00 | 0.10 | 0.75 |  |  |  |  |  |
|  |  | s(Gut fullness) | 2.08 | 0.78 | 0.65 |  |  |  |  |  |
|  |  | Basin | 4.00 | 1.01 | 0.42 |  | 4.00 | 3.16 | 0.03 |  |



Figure S1. Decision tree describing the classification process of microparticles found in the gastro-intestinal tract of herring using both quantitative FTIR-data and qualitative visual information.



Figure S2. Schematic representation of the model used to predict microplastic ingestion in Baltic herring.

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Figure S3. Consumption rates (left axis) and clearance rates (right axis) as a function of *Calanus finmarchicus* abundance. The values are based on the data presented in Fig. 5, Varpe and Fiksen (2010) and adjusted for fish with average body weight of 35 g.

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**Figure S4.** Modeled MP burden (MP ind-1) in the first ten simulation runs for 48 h. Observe that values are stabilized at the end of the simulation; these values are used to represent intrapopulation variability. Three out of ten individuals contain no MP.



Figure S5. Diagnostic plot for the zero inflated poisson GAM model. The quantile-quantile plot shows the distribution of the deviance residuals as a function of residual quantiles from a theoretical distribution. Black points represent observations. Grey lines are simulated residuals from randomly generated zero inflated data. Observations that distribute close to the theoretical distribution (red line) and are within the bounds of the simulated data indicate appropriate model specification.



Figure S6. Diagnostic quantile-quantile plots for the confirmatory GAM-analysis (Table S6). The quantile-quantile plot shows the distribution of the deviance residuals as a function of residual quantiles from a theoretical distribution. Black points represent observations. Grey lines are simulated residuals from randomly generated data. Grey bands are 95% confidence intervals. Observations that distribute close to the theoretical distribution (red line) and are within the bounds of the simulated data indicate appropriate model specification. Each panel refers to a specific model with one of the HOC-groups as the dependent variable: PCBs (A), BDEs (B), DDs (C), HBCD (D) and HCB (E).

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