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1 **Hydrophobic organic contaminants are not linked to microplastic uptake in**

2 **Baltic Sea herring**

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20

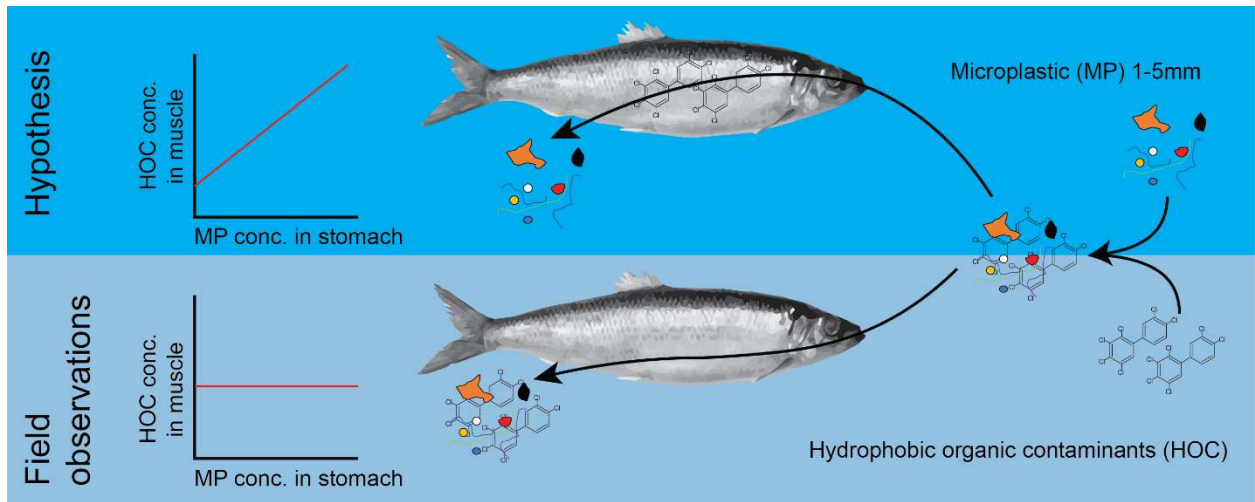
21 **Abstract**

22 It is commonly accepted that microplastic (MP) ingestion can lead to lower food intake and
23 bioaccumulation of hydrophobic organic contaminants (HOCs) in aquatic organisms. However,
24 causal links between MP and contaminant levels in biota are poorly understood and *in situ* data
25 are very limited. Here, we investigated whether HOC concentrations in herring muscle tissue
26 (*Clupea harengus membras*) are related to MP ingestion using fish caught along the West coast
27 of the Baltic Sea. The MP occurrence exhibited a large geographic variability, with MP found in
28 22.3% of the fish examined. The population average was 1.0 MP ind⁻¹; however, when only
29 individuals containing MP were considered, the average MP burden was 4.4 MP ind⁻¹. We also
30 found that MP burden decreased with reproductive stage of the fish but increased with its body
31 size. To predict MP abundance in fish guts, we constructed a mass-balance model using literature
32 data on MP in the water column and physiological rates on ingestion and gut evacuation for
33 clupeids of a similar size. The model output was in agreement with the observed values, thus
34 supporting the validity of the results. Contaminant concentrations in the muscle tissue were
35 unrelated to the MP levels in fish, suggesting a lack of direct links between the levels of HOCs
36 and MP ingestion. Thus, despite their ubiquity, MP are unlikely to have a measurable impact on
37 food intake or the total body burden of hydrophobic contaminants in Baltic herring.

38

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

40



41

42

43 **Introduction**

44 Plastic debris, including microplastics (MP < 5 mm), can be ingested by aquatic animals across
45 several trophic levels (Lusher et al. 2013, 2015, Cole et al. 2013). Due to the importance of
46 commercial fish and shellfish species for human consumption, the ingestion and presence of MP
47 in these animals has become a matter of concern (EFSA Panel on Contaminants in the Food Chain
48 (CONTAM) 2016). To address this concern and to provide a quantitative assessment of MP
49 ingestion in various fish species, an active research is ongoing (Lusher et al. 2013, Foekema et al.
50 2013, Rummel et al. 2016, Budimir et al. 2018, Beer et al. 2018).

51
52 A commonly held paradigm states that MP ingestion can lead to decreased nutritional status
53 (Cole et al. 2015, Ogonowski et al. 2016) and bioaccumulation of hydrophobic organic chemicals
54 (HOCs) (Oliveira et al. 2012, Besseling et al. 2013, Rochman et al. 2013, Wardrop et al. 2016) that
55 sorb to the MP particles in the water and desorb in the gut lumen (Mato et al. 2001, Rusina et al.
56 2010, Rochman et al. 2014). However, some experimental and modeling studies indicate that
57 plastic polymers could also have a net cleaning effect acting as passive samplers while in the
58 digestive system and thereby relieve the animals of HOCs (Gouin et al. 2011, Herzke et al. 2016,
59 Koelmans et al. 2016, Mohamed Nor and Koelmans 2019). The relative importance of
60 microplastics as vectors for contaminant transport remains unresolved, possibly also due to the
61 lack of field data linking HOC concentrations in biota to ingested MP.

62
63 Here, we studied MP ingestion by Baltic Sea herring (*Clupea harengus membras* L.), a
64 commercially exploited fish and a keystone species in the Baltic food web. Being facultative

65 pelagic filter-feeders (Huse and Toresen 1996), herring stand a high risk of ingesting MP along
66 with zooplankton prey and hence accumulating MP-associated contaminants. It is also a sentinel
67 species in the Swedish National Monitoring Program for Contaminants in Marine Biota and, thus,
68 a potential indicator species for MP monitoring in the Baltic Sea (Beer et al. 2018).

69
70 If MP ingestion indeed contributes significantly to HOC bioaccumulation in contaminated
71 environments, then one would see a positive correlation between the amount of MP ingested
72 over time and HOC concentrations in the herring tissues. However, there are no reliable methods
73 to estimate accumulated MP exposure using field samples, because MP do not accumulate to
74 any significant extent in the fish digestive system (Lusher et al. 2013, Jovanović 2017). Although
75 gut contents reflect only a recent ingestion history (Ahlbeck et al. 2012), the MP burden
76 determined by gut content analysis is commonly used as a reflection of the feeding habits and
77 habitats of the fish. Another area of concern with respect to the interpretation of MP counts in
78 environmental samples, including fish guts, is analytical accuracy and reliability of MP extraction
79 and determination (Dehaut et al. 2016). Therefore, to increase the reliability of the MP gut
80 content data, it is important to verify whether the recorded MP body burden is within ecologically
81 plausible rates of ingestion and gut evacuation. To compare the observed MP abundance in the
82 fish gut with the intake that can be expected given the MP abundance in the water column, and
83 gut evacuation that can be expected given the food intake, a mass-balance modelling approach
84 can be used. We applied such modeling in this study using literature-derived parameters on
85 clupeid feeding and food processing as well as ambient MP concentrations, to estimate MP
86 burden in the herring with a body size similar to those in our collection. We also evaluated
87 whether HOC concentrations in the fish muscle were related to the weight-specific MP gut
88 content of the same individual.

89

90 **Materials and Methods**

91 *Fish collection and sample characteristics*

92 The Baltic herring used for our analyses were collected by the Swedish National Monitoring
93 Program for Contaminants in Marine Biota conducted by the Swedish Museum of Natural History
94 (Stockholm, Sweden). To avoid possible bias by known point sources, we randomly selected 130
95 specimens that had been collected at thirteen reference monitoring stations (Figure 1), thus
96 covering a sufficiently large geographical area that would provide a representative range of HOC
97 and MP exposure for the analysis.

98

99 The sex ratio of the selected fish was approximately 50:50 and uniform across sampling sites. The
100 individuals were 3-7 years old, with a total length of 173 ± 18 mm and body weight 35 ± 12 g
101 (mean \pm SD). The reproductive phase determined by gametocytic maturity was classified on a
102 five-degree scale according to Bucholtz et al. (2008) and included deformed gonads (stage 1),
103 post spawned individuals (stage 2), juveniles (stage 3), individuals with developing gonads (stage
104 4) and fish with mature gonads (stage 5). Each fish was dissected, and the muscle tissues taken
105 from the middle dorsal muscle layer were used for HOC analysis, whereas the entire
106 gastrointestinal tract (GIT) was used for the MP analysis. All sampling was performed according
107 to standard procedures (TemaNord 1995). After dissecting, each individual GIT was packed in
108 aluminum foil to avoid cross-contamination. All GIT samples were immediately frozen at -20 °C
109 and stored until MP analysis at the Department of Environmental Science and Analytical
110 Chemistry, Stockholm University, Sweden.

111

112 *MP quantification in the gastrointestinal tract of fish*

113 Each GIT was placed in a glass Petri dish, opened with surgical scissors, and rinsed with deionized,
114 particle-free water. Using a stereo microscope, the bolus was examined, and any items
115 resembling MP were extracted by stainless steel pincers and transferred to clean Eppendorf
116 tubes. The appearance of the putative MP was recorded and each particle was categorized
117 according to its shape (fiber or fragment) and color. Hereafter, the number of MP per individual
118 fish is referred to as *MP burden*.

119

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

131 The following polymer identification scheme was applied. First, to identify whether the putative
132 MP were synthetic polymers, we followed the recommendations of Norén (2007) and Hidalgo-

133 Ruz et al. (2012). Particles 1-5 mm in diameter were recorded and classified as MP, if all the
134 following criteria were met: (i) uniform, unnaturally bright or of an unnatural color, (ii) lack of
135 organic structures, and (iii) uniform diameter over the entire length of a fiber. Second, to test the
136 accuracy of the visual identification, a random subset of 16 samples containing putative MP (i.e.,
137 the gut contents of 16 individual fish, containing in total 26 microparticles) was analyzed using
138 Fourier transform infrared spectroscopy (FTIR). Third, MP-validation was performed by
139 comparing the sample spectra to a published reference database (Primpke et al. 2018). The Hit
140 Quality Index (HQI) was used to determine whether a sample spectrum matched any spectra in
141 the database. The HQI-threshold for a match was set at 70% similarity (Thompson et al. 2004). A
142 detailed description of the data preparation of sample spectra is provided in Supporting
143 Information 1.1.

144

145 *FTIR analysis*

146 The infrared spectra were recorded at 4 cm^{-1} resolution with a Bruker Vertex 70 FTIR instrument
147 that was equipped with a Bruker Platinum attenuated reflection (ATR) unit. Data were recorded
148 on both sides of the center burst of the interferogram during forward and backward movement
149 of the interferometer mirror. A zero filling factor of 2 was used and the spectra were apodized
150 with a Blackman-Harris 3-term function. The spectra for samples 1 – 6 (Supporting Information
151 Table S 1) were recorded using a HgCdTe detector, 100 sample scans were recorded and the
152 scanning time was 22 sec. The spectra for samples 7 – 26 (Supporting Information Table S 1) were
153 recorded using a DTGS detector, 364 sample scans were recorded within 300 s scanning time.
154 Individual particles were placed on the diamond crystal of the ATR unit and pressed onto the
155 crystal with a piston. Prior to each measurement the crystal was cleaned with 99% ethanol.

156

157 *Controls and blanks*

158 To prevent contamination by airborne particles during the examination, the dissections were
159 performed under a Fumex local extractor (Wesch et al. 2016); each sample being analyzed for 10
160 min. A Petri dish filled with filtered deionized water was placed next to a test sample to serve as
161 a blank for the quantification and characterization of potential contamination during the analysis.
162 When working with samples, a cotton lab coat and gloves were used: moreover, the type and
163 color of clothing were recorded to enable contamination back-tracing. All procedural blanks
164 contained particles (mainly single fibers) of unknown origin. However, all these particles were <
165 1 mm and thus did not contribute to the MP counts used in the statistical analysis. If quantifiable
166 amounts of blank contamination with particles > 1 mm were to be found, such samples would be
167 excluded from any further analyses.

168

169 *Chemical analysis*

170 Following the guidelines of the Swedish National Monitoring Program for Contaminants in
171 Marine Biota, the muscle samples were analyzed for polychlorinated biphenyls (PCB 28, 52,
172 101, 118, 138, 153 and 180), organochlorine pesticides (DDE, DDD, DDT, HCB, AHCH, BHCH, and
173 Lindane) and polybrominated flame retardants (BDE 28, 47, 99, 100, 153, 154 and HBCD). For
174 most compounds, 10 g of muscle tissue from individual fish were used, whereas 1 g samples of
175 muscle tissue from 10 individuals were pooled for a few analytically challenging compounds. An
176 overview of the analyzed contaminants and their average concentrations in herring muscle

177 tissue are provided in Table 1, while details of the analytical procedures and quality assurance
178 are provided elsewhere (Bignert et al. 2016).

179

180 *Data analysis and statistics*

181 *Relationships between biological factors, geography and ingested microplastic*

182 We used generalized additive models (GAM) in package *mgcv* to examine relationships between
183 specific biological variables (*weight, gut fullness, age* and *reproductive phase*) and *MP burden*;
184 *sea basin* was used as a random factor in the model since the inclusion of this term lowered the
185 Akaike Information Criterion from 323 to 270. The model was specified as:

186

187 $MP\ burden = \beta s(weight) + s(gut\ fullness) + s(age) + s(reproductive\ phase) + random(sea\ basin) +$
188 ϵ

189

190 The multicollinearity between the explanatory variables was evaluated as low (< 0.38) using
191 concavity measures (Amodio et al. 2014) calculated by the *mgcv*-package. Due to the
192 overrepresentation of zeros in the data (overdispersion) for the *MP burden*, the model was run
193 using zero-inflated Poisson error structures. Model performance was assessed using residual
194 plots. Differences in the *MP burden* between the basins were tested using Permanova with
195 *station* nested within *basin* as a random factor (Anderson 2001). The significance level was set at
196 $\alpha = 0.05$; all statistical analyses were conducted in R 3.5.0 (R Core Team 2014).

197

198 *Relationships between HOCs and ingested microplastic*

199 Maximum-likelihood Factor Analysis with Varimax rotation was used to assess the degree of
200 association between the chemical variables and weight-specific MP burden in the GIT. Prior to
201 the analysis, Bartlett's test of sphericity was performed to confirm patterned relationships
202 between the variables and was statistically significant ($\chi^2_{15} = 176$, $p < 0.0001$). A scree plot was
203 used to determine the number of factors to retain, and factor loadings > 0.7 were considered
204 statistically significant (MacCallum et al. 2001). When measured values were below the limit of
205 quantification (LOQ), they were imputed by LOQ divided by the square root of two (Succop et al.
206 2004). The analyzed chemical concentrations were summed and grouped into their respective
207 contaminant groups (PCBs, PBDEs and organochlorine pesticides).

208

209 *Modeling plastic ingestion by herring*

210 To evaluate whether the observed MP burden could be predicted using ambient MP abundance
211 data and food processing rates, we modeled the ingestion of MP using literature-derived
212 parameters on food uptake, egestion, and MP abundance in the study area. The rationale is that
213 observed MP abundance in the gut would reflect average exposure levels assuming that (1) MP
214 concentrations are fairly homogeneous in the outer coastal areas (Gorokhova 2015, Gewert et
215 al. 2017), which are the main feeding grounds of herring (Flinkman et al. 1998), (2) the MP
216 abundance in the water column, where the fish feed, is similar to that at the surface, where the
217 data on the relevant size fraction of MP (1- 5 mm) were collected; (3) MP ingestion by herring is
218 non-selective and thus proportional to the MP abundance in the water, and (4) gut evacuation
219 rates are non-discriminatory, i.e., MP are egested at the same rate as prey remains. Then, the

220 MP burden ($MP \text{ ind}^{-1}$) at any given time, t , can be written as the mass balance between the uptake
221 and loss rates (Eq. 1):

222

$$223 \quad MP_t = MP(t - dt) + (IR - Eg) dt, \quad (1)$$

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

$$226 \quad IR = CMP \times CR \quad (2)$$

227 and

$$228 \quad ER = GER \times MP_t, \quad (3)$$

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

232

233 We used literature data to parameterize the model (Supporting Information Figure S 1, Table S
234 3). The MP concentrations in the target size range (1-5 mm) from surface waters in the outer
235 Stockholm archipelago (Gewert et al. 2017) were used as CMP values. Clearance rates were
236 estimated using reported feeding rates for North Sea herring on *Calanus finmarchicus*, a
237 copepod of similar size as the microplastics considered here, and the main prey for herring
238 (Varpe and Fiksen 2010) (see Supporting Information 2.1 for the calculation of CR). As published
239 gut evacuation rates for adult herring were not available, we used experimental values
240 reported for other clupeids of similar size, European pilchard (*Sardina pilchardus*) (Costalago

241 and Palomera 2014) and South American pilchard (*Sardinops sagax*) (van der Lingen 1998),
242 which have similar feeding ecology and physiology as Baltic herring (Collard et al. 2017). The
243 physiological rates used in the model corresponded to the average size of our fish.

244
245 The model was implemented using STELLA® ver. 9.4.1 software (iSee systems, Inc. Lebanon, NH,
246 U.S.A.) to estimate MP burden (MP ind⁻¹) dynamics in a fish population at a given MP abundance.
247 The intrapopulation variability was simulated using a Monte Carlo generator with 1000
248 permutations (details on the simulation settings are provided in Supporting Information 2.2). To
249 validate the model, we compared the simulated data distribution from the model to the field
250 data using descriptive statistics, χ^2 , and the two-sample Cramér-von Mises tests.

251

252 **Results**

253 *Observed MP burden*

254 Particles identified by visual inspection as MP were found in 44 out of the 130 individuals (33.8%;
255 range: 0 to 51 pieces of plastic fiber or fragments ind⁻¹). In these 44 individuals, the mean
256 abundance was 7.8 ± 12.2 particles ind⁻¹ (\pm SD). The dominant type of the MP were fibers of
257 various colors (87.6%), while fragments were less frequent (12.4%). However, only 38.5 % of the
258 putative MP were classified as either synthetic or semi-synthetic (viscose) by FTIR and 38.5%
259 were classified as being of natural origin (e.g. chitin, fur and cellulose); 23 % could not be
260 identified (Supporting information Table S 1). None of the samples exclusively matched the most
261 commonly found polymers in the environment; polyethylene (PE), polypropylene (PP),
262 polystyrene (PS), polyethylene terephthalate (PET) and polyvinylchloride (PVC).

263

264 After correcting for the proportion of the misclassified samples, only 17 individuals containing
265 MP remained (13.1 %; range: 0 to 20 MP), with a mean abundance of $4.5 \text{ MP ind}^{-1} \pm 5.3$. When
266 all examined individuals were considered, the population average was 0.9 MP ind^{-1} , with the 95%
267 bootstrap confidence interval ranging $0.5 - 1.6 \text{ MP ind}^{-1}$. The variation in the MP burden between
268 the stations and basins was high (Figure 2, Supporting information Table S 2) and no significant
269 differences in the MP burden between the basins were found (*station* nested within *basin* as a
270 random factor, pseudo $F_{4,117} = 0.9$, $p = 0.49$).

271

272 *Predicted vs. observed MP burden and frequency of occurrence*

273 The model predicted that 81% of fish contained MP, with a mean MP burden of 4.7 MP ind^{-1} ;
274 these values were about five times as high as the observed values. The ranges of the frequency
275 distributions for the simulated and observed values were overlapping, although the field
276 observations were more strongly skewed towards zero values compared to the model
277 prediction (Figure 3 A; Supporting Information Table S 4). The difference between the
278 distributions was statistically significant (Cramér-von Mises $T = 186$, $p < 0.0001$). However,
279 when zero values were excluded, the distributions, albeit still significantly different (Cramér-
280 von Mises $T = 10.6$, $p < 0.01$), became more similar (Figure 3 B; Supporting Information Table S
281 4), indicating that much of the difference between the distributions was driven by the
282 significantly higher proportion of zero observations in the field data ($\chi^2 = 219.5$, $p < 0.0001$).

283

284 *Linkage between MP intake and HOCs*

285 We found no relationship between the weight-specific MP burden and the concentration of any
286 of the HOCs (Figure 4). Together, the two factors explained a cumulative variance of 84.4%
287 (Supporting Information Table S 5). As a variable, weight-specific MP burden loaded weakly and
288 negatively (-0.14) on the first axis and moderately positive (0.58) on the second axis. In contrast,
289 the organochlorine pesticides and PBDEs loaded significantly and positively on the first axis, while
290 the PCBs loaded moderately positive (0.56) on the first and significantly positive (0.82) on the
291 second axis. Hence, no contaminant group had loadings clustering with those for the weight-
292 specific MP burden.

293

294 *Biological factors related to MP burden*

295 The MP burden was positively and nearly linearly related to fish *body weight* (GAM, $\chi^2 = 13.1$, p
296 < 0.01 , Figure 5 A). In contrast, a negative effect was found for *reproductive phase*, where MP
297 burden was significantly lower in fish that had reached sexual maturity (GAM $\chi^2 = 16.4$, $p < 0.01$,
298 Figure 5 B). *Gut fullness* only had a negative effect on MP burden when the GIT was empty of
299 food items (GAM $\chi^2 = 39.4$, $p < 0.0001$, Figure 5 C) while *Age* displayed a weak negative
300 relationships with MP burden (GAM $\chi^2 = 16.6$, $p < 0.001$, Figure 5 D).

301

302 **Discussion**

303 *Microplastics are common but not abundant in herring guts*

304 Microplastics (mostly fibers of synthetic and semi-synthetic origin) were found in about 20% of
305 the fish. While these values are in good agreement with those reported for herring by Beer et al.

306 (2018) for the central Baltic Sea (20% containing MP, with 93% fibers), other studies report
307 considerably lower MP frequency of occurrence and fiber contribution to total MP in herring.
308 Both Foekema et al. (2013) and Rummel et al. (2016) found plastics in only 2% of herring samples
309 from the North Sea and the Southern Baltic Sea, with fibers accounting for less than 10% of MP.
310 Having excluded fibers from their analyses, Budimir et al. (2018) reported a frequency of
311 occurrence as low as 1.8% in herring from the northern Baltic Sea. These discrepancies between
312 different studies could be related to differences in fish size and gut fullness. For example,
313 Foekema et al. (2013) used fish that were considerably larger (>200 mm total length) which most
314 likely already had switched from filter feeding to raptorial feeding on larger prey (Huse and
315 Toresen 1996). This change in feeding mode would result in a lower ingestion rate of
316 zooplankton-sized plastic particles and thus in a lower overall MP burden. In the study of Rummel
317 et al. (2016), many fish stomachs were empty, which probably was related to arrested feeding in
318 concert with spawning, and, possibly, stress-induced gut evacuation caused by the fish sampling
319 (Wilkins 1967, Vinson and Angradi 2011). This lends further support to our findings that MP
320 burden increases with fish size (Beer et al. 2018) and decreases with reproductive phase (Stacey
321 and Hourston 1982). In addition, one would expect the amount of ingested MP to scale with the
322 absolute size of bolus or gut fullness. However, since this relationship was weak (Figure 5 C), our
323 findings only partly support this expectation. One possible explanation for this could be slower
324 egestion of MP compared to prey, similar to the selective retention of plastic fibers in amphipods
325 (Au et al. 2015) and fragments in cladocerans (Ogonowski et al. 2016), which would result in a
326 temporary accumulation of MP in the fish gut and obscure the expected positive relationship
327 between the gut fullness and MP burden. While fish size appears to be the strongest covariable
328 for standardizing gut MP content, gut fullness was also influential, particularly for fish with empty
329 guts, which may occur during fasting periods (Darbyson et al. 2003). Although the effect of *Age*

330 also was statistically significant, the effect was not particularly strong and most probably of low
331 biological importance.

332

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

353

354 *No correlation between weight-specific MP burden and HOCs*

355 The transfer of hydrophobic contaminants from ingested plastics to biota has been described as
356 the so-called "Trojan horse" effect (Cole et al. 2011). While this transfer has been demonstrated
357 under laboratory conditions (Besseling et al. 2013, Rochman et al. 2013, Browne et al. 2013, Batel
358 et al. 2016), recent modelling studies indicate that natural sources are much more important
359 than MP in explaining HOC bioaccumulation patterns in aquatic organisms (Koelmans 2015,
360 Koelmans et al. 2016, Mohamed Nor and Koelmans 2019). We did not find any correlation
361 between HOC concentrations in herring muscle and MP burden, although it could be argued that
362 omitting small MP (< 1 mm) from our analysis, could have biased the results. Indeed, by focusing
363 on the larger MP, we ignored the potentially important influence of a higher total surface area
364 and thus higher HOC desorption rates (Hendriks et al. 2001, Hartmann et al. 2017). However, the
365 ingestion of such small particles by fish of this size is rather unlikely, because filter-feeding herring
366 have a relatively low capacity to retain small particles due to their rather wide gill raker spacing
367 (Gibson 1988, Collard et al. 2017) and actively avoid smaller prey while feeding raptorially (Aro
368 et al. 1989, Casini et al. 2004). In fact, this line of reasoning has been supported by several other
369 studies reporting a predominant retention of MP of > 1 mm by similarly-sized herring (Lenz et al.
370 2016, Collard et al. 2017, Beer et al. 2018). Moreover, given the short residence time (Grigorakis
371 et al. 2017) of ingested plastics and the slow desorption kinetics of many HOCs, the lack of
372 correlation between the MP and organic contaminants is rather expected and in line with other
373 reports for fish and other aquatic animals (Herzke et al. 2016, Rehse et al. 2018, Kleinteich et al.
374 2018).

375

376 Causality is difficult to prove using environmental samples, where many different parameters
377 may affect contaminant body burden of an organism (Hartmann et al. 2017), including various
378 biotic factors that have significant effects on both MP (this study) and HOC levels (Persson et al.
379 2013, Silva Barni et al. 2014). However, our findings suggest that there is no tenable relationship
380 between the MP intake and tissue contaminant concentrations in the Baltic herring (Figure 4).
381 Similarly, no correlation has been found between the amount of ingested plastic and HOC
382 concentrations in northern fulmars (*Fulmarus glacialis*) from the Norwegian coast (Herzke et al.
383 2016), even though the birds had ingested much larger amounts of plastic and their gut passage
384 time for plastic debris is several orders of magnitude longer than in herring (Ryan 2015). This lack
385 of relationship is also supported by the relatively constant MP burden observed in Baltic herring
386 over the past three decades (Beer et al. 2018), while muscle concentrations of HOCs have
387 decreased significantly (Bignert et al. 2016). The mass-balance model indicates that our
388 measurements of the MP burden are ecologically plausible given the currently reported
389 abundances of MP in the Baltic surface water, thus supporting the reliability of the MP burden
390 estimates in the Baltic herring reported here and in other studies and providing confidence in the
391 methods employed. Taken together, these findings contrast the currently held paradigm that
392 microplastics are an important source of HOCs in aquatic organisms (Mato et al. 2001, Rochman
393 et al. 2013).

394

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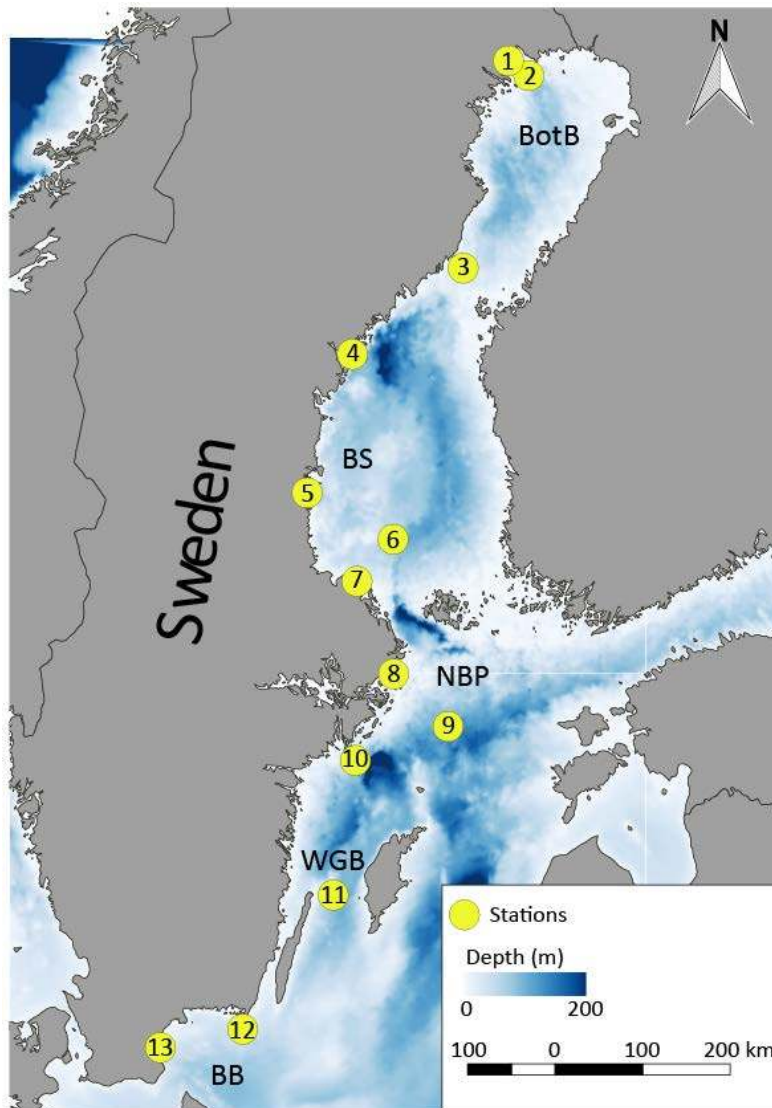
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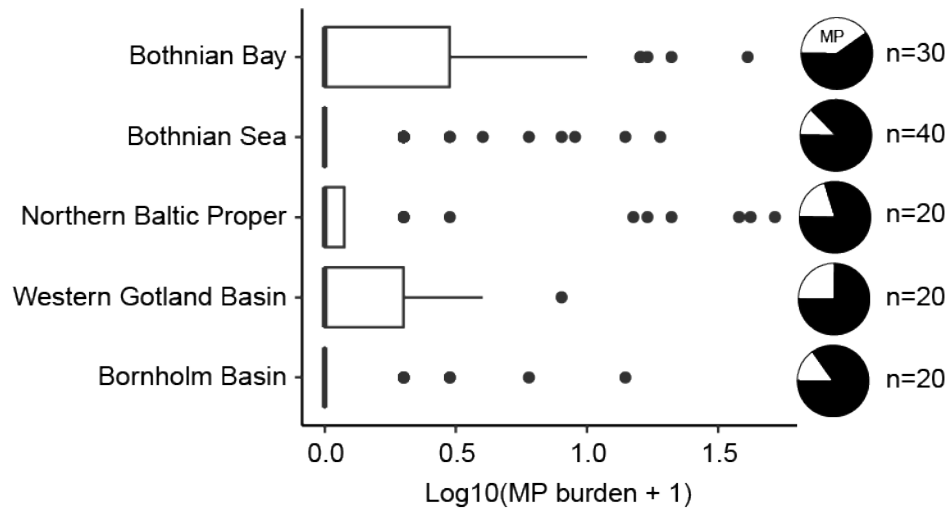
607 **Figures and tables**



608

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

615



616

617 **Figure 2.** Boxplot of Log10-transformed MP abundance in the gastrointestinal tract (GIT) of
618 herring per basin ordered from north to south. Data are presented as medians (vertical lines),
619 inter quartile range, IQR (boxes), 1.5 IQR (whiskers) and outliers (points) being > 1.5 IQR. The
620 black slices of the pie charts indicate the proportion of examined herring with no MP in the GIT.

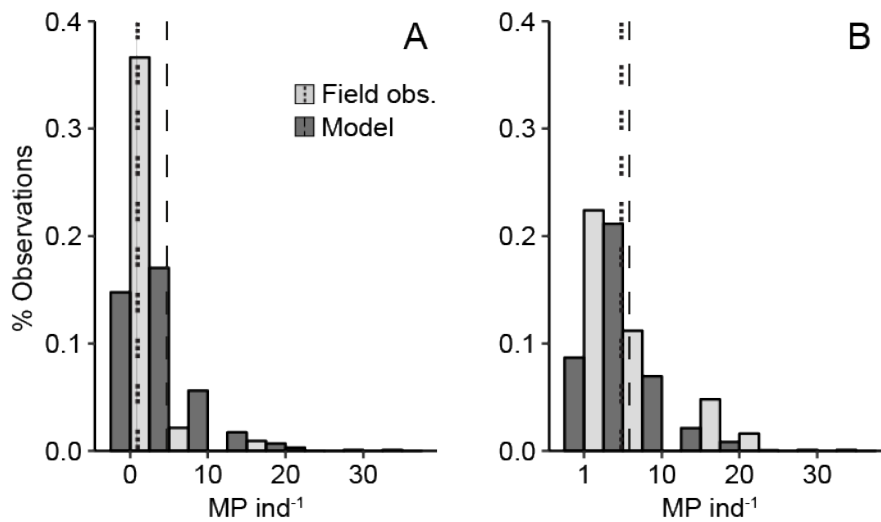


Figure 3. Frequency distribution of the MP burden based on the model simulations (dark grey bars) and field observations (light grey bars). Panel A shows the entire dataset and panel B presents only fish with MP in the GIT (i.e., the non-zero values). The dashed vertical lines indicate the mean values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

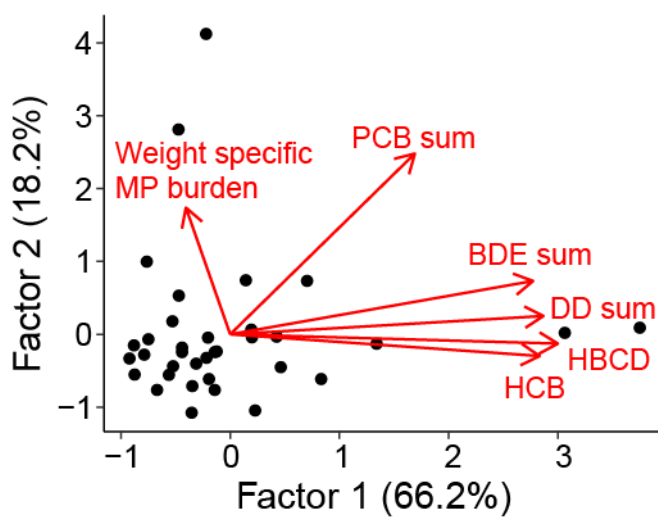


Figure 4. Factor scores (axes) and loadings (arrows) of contaminants (HBCD, HCB and the sum of PCBs, BDEs and DDs) and weight-specific MP burden.

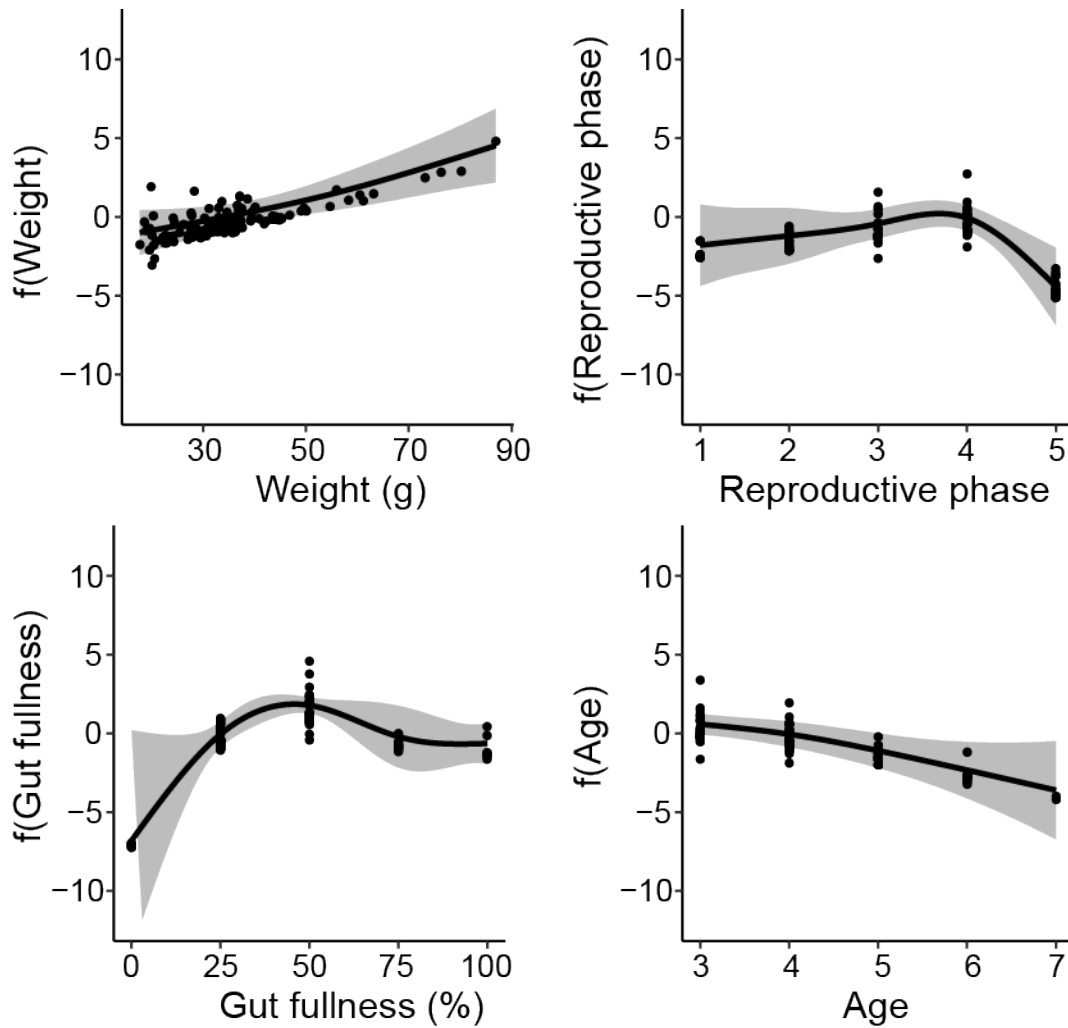


Figure 5. Generalized additive models (GAMs) showing partial response curves for the explanatory biological variables: *body weight* (A), *reproductive phase* (B), *gut fullness* (C) and *age* (D). The classes for *reproductive phase* correspond to: 1 = deformed gonads, 2 = post spawned, 3 = juvenile, 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. Grey bands indicate 95% confidence interval for each curve.

Table 1. Overview of the HOCs in herring muscle tissue and descriptive statistics of their concentrations ($\mu\text{g g}^{-1}$ fish muscle). SD – standard deviation.

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

