

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

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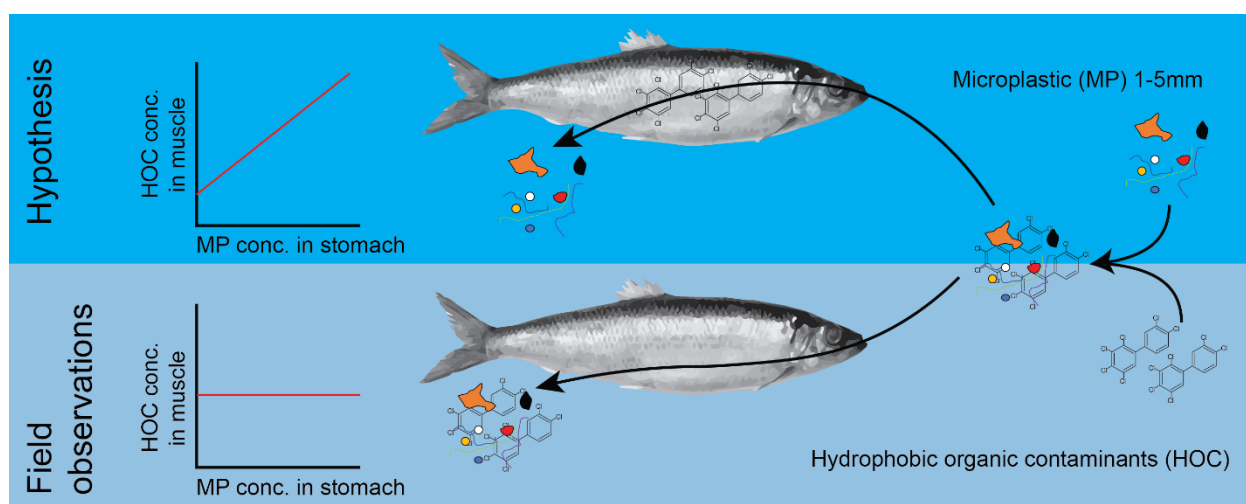
14 **Abstract**

15 It is commonly accepted that microplastic (MP) ingestion can lead to lower food intake and
16 bioaccumulation of hydrophobic organic contaminants (HOCs) in aquatic organisms. However,
17 causal links between MP and contaminant levels in biota are poorly understood and *in situ* data
18 are virtually non-existent. Here, we investigated whether HOC concentrations in herring muscle
19 tissue (*Clupea harengus membras*) are related to MP ingestion using fish caught along the West
20 coast of the Baltic Sea. The MP occurrence exhibited a large geographic variability, with MP found
21 in 28% of the fish examined. The population average was 2.4 MP ind⁻¹; however, when only
22 individuals containing MP were considered, the average MP burden was 8.4 MP ind⁻¹. We also
23 found that MP burden decreased with reproductive stage of the fish but increased with its body
24 size. To predict MP abundance in fish guts, we constructed a mass-balance model using literature
25 data on MP in the water column and physiological rates on ingestion and gut evacuation for
26 clupeids of a similar size. The model output was in agreement with the observed values, thus
27 supporting the validity of the results. Contaminant concentrations in the muscle tissue were
28 unrelated to the MP levels in fish, suggesting a lack of direct links between the levels of HOCs
29 and MP ingestion. Thus, despite their ubiquity, MP are unlikely to have a measurable impact on
30 food intake or the total body burden of hydrophobic contaminants in Baltic herring.

31

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

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34

35

36 **Introduction**

37 Plastic debris, including microplastics (MP < 5 mm), can be ingested by various aquatic organisms
38 across several trophic levels (Lusher et al. 2013, 2015, Cole et al. 2013). Due to the importance of
39 commercial fish and shellfish species for human consumption, the ingestion and presence of MP
40 in these animals has become a matter of concern (EFSA Panel on Contaminants in the Food Chain
41 (CONTAM) 2016). To address this concern and to provide a quantitative assessment of MP
42 ingestion in various fish species, an active research is ongoing (Lusher et al. 2013, Foekema et al.
43 2013, Rummel et al. 2016, Budimir et al. 2018, Beer et al. 2018).

44 A commonly held paradigm states that MP ingestion can lead to decreased nutritional status (Cole
45 et al. 2015, Ogonowski et al. 2016) and bioaccumulation of hydrophobic organic chemicals
46 (HOCs) (Oliveira et al. 2012, Besseling et al. 2013, Rochman et al. 2013, Wardrop et al. 2016)
47 that sorb to the MP particles in the water and desorb in the gut lumen (Mato et al. 2001, Rusina et
48 al. 2010, Rochman et al. 2014). However, some experimental and modeling studies indicate that
49 plastic polymers could also have a net cleaning effect acting as passive samplers while in the
50 digestive system and thereby relieve the animals of HOCs (Gouin et al. 2011, Herzke et al. 2016,
51 Koelmans et al. 2016). The relative importance of microplastics as vectors for contaminant
52 transport remains unresolved, possibly also due to the lack of field data linking HOC
53 concentrations in biota to ingested MP.

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

60 If MP ingestion indeed contributes significantly to HOC bioaccumulation in contaminated
61 environments, then one would see a positive correlation between the amount of MP ingested over
62 time and HOC concentrations in the herring tissues. However, there are no reliable methods to
63 estimate accumulated MP exposure using field samples, because MP do not accumulate to any
64 significant extent in the fish digestive system (Lusher et al. 2013, Jovanović 2017). Although gut
65 contents reflect only a recent ingestion history (Ahlbeck et al. 2012), the MP burden determined
66 by gut content analysis is commonly used as a reflection of the feeding habits and habitats of the

67 fish. Another area of concern with respect to the interpretation of MP counts in environmental
68 samples, including fish guts, is analytical accuracy and reliability of MP extraction and
69 determination (Dehaut et al. 2016). Therefore, to increase the reliability of the MP gut content
70 data, it is important to verify whether the recorded MP body burden is within ecologically plausible
71 rates of ingestion and gut evacuation. To compare the observed MP abundance in the fish gut with
72 the intake that can be expected given the MP abundance in the water column, and gut evacuation
73 that can be expected given the food intake, we applied a mass-balance modelling. In this model,
74 using literature-derived parameters on clupeid feeding and food processing as well as ambient MP
75 concentrations, we estimated MP burden in the herring with the body size similar to those in our
76 collection. Finally, we evaluated whether HOC concentrations in the fish muscle were related to
77 the weight-specific MP gut content of the same individual.

78

79 **Materials and Methods**

80 *Fish collection and sample characteristics*

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

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

99

100 *MP quantification in the gastrointestinal tract of fish*

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

107 To relate HOC concentration in the muscle tissue and MP intake by fish, the fish size must be
108 taken into account when expressing MP counts. Moreover, the bolus size varied considerably
109 among the individual fish and geographical areas (Supporting Information Table S1), indicating
110 variability in feeding activity shortly before sampling and/or gut evacuation that might have been
111 related to stress during the sampling. To account for this variability and the corresponding
112 variation in the MP burden, we normalized the individual MP counts to its gut fullness; the latter
113 was assessed by visual observation on a five-step semi-quantitative scale: 0 (empty, no food items),
114 0.25, 0.5 0.75 or 1 (full). The obtained values were further normalized to the individual body
115 weight and termed *weight-specific MP burden* [number of MP / (gut fullness × body weight) (g
116 wet weight)]. This allowed relating HOC concentrations in the fish to the expected MP burden in
117 the GIT on a weight basis.

118 To identify whether the putative MP were synthetic polymers, we followed the recommendations
119 of Norén (2007) and Hidalgo-Ruz et al. (2012). Particles 1-5 mm in diameter were recorded and
120 classified as MP, if all the following criteria were met: (i) uniform, unnaturally bright or of an
121 unnatural color, (ii) lack of organic structures, and (iii) uniform diameter over the entire length of
122 a fiber. To test the accuracy of the visual identification, a random subset of 20 samples containing
123 MP (i.e., the gut contents of 20 individual fish) was analyzed using the hot needle test (De Witte
124 et al. 2014).

125

126 *Controls and blanks*

127 To prevent contamination by airborne particles during the examination, the dissections were
128 performed under a Fumex local extractor (Wesch et al. 2016); each sample being analyzed for 10
129 min. A Petri dish filled with filtered deionized water was placed next to a test sample to serve as a

130 blank for the quantification and characterization of potential contamination during the analysis.
131 When working with samples, a cotton lab coat and nitrile gloves were used: moreover, the type
132 and color of clothing were recorded to enable contamination back-tracing. All procedural blanks
133 contained plastic particles (mainly single fibers) of unknown origin. However, all these particles
134 were < 1 mm and thus did not contribute to the MP counts used in the statistical analysis. If
135 quantifiable amounts of blank contamination with particles > 1 mm were to be found, such samples
136 would be excluded from any further analyses.

137

138 *Chemical analysis*

139 Following the guidelines of the Swedish National Monitoring Program for Contaminants in
140 Marine Biota, the muscle samples were analyzed for polychlorinated biphenyls (PCB 28, 52,
141 101, 118, 138, 153 and 180), organochlorine pesticides (DDE, DDD, DDT, HCB, AHCH,
142 BHCH, and Lindane) and polybrominated flame retardants (BDE 28, 47, 99, 100, 153, 154 and
143 HBCD). For most compounds, 10 g of muscle tissue from individual fish were used, whereas 1 g
144 samples of muscle tissue from 10 individuals were pooled for a few analytically challenging
145 compounds. An overview of the analyzed contaminants and their average concentrations in
146 herring muscle tissue are provided in Table 1, while details of the analytical procedures and
147 quality assurance are provided elsewhere (Bignert et al. 2016).

148

149 *Data analysis and statistics*

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

151 We used generalized additive models (GAM) in package *mgcv* to examine relationships between
152 specific biological variables (*weight*, *gut fullness*, *age* and *reproductive phase*) and *MP burden*;
153 *sea basin* was used as a random factor in the model since the inclusion of this term lowered the
154 Akaike Information Criterion from 570 to 417. The model was specified as:

155

$$156 \text{MP burden} = \beta s(\text{weight}) + s(\text{gut fullness}) + s(\text{age}) + s(\text{reproductive phase}) + \text{random}(\text{sea basin})$$

157 + ϵ

158

159 The multicollinearity between the explanatory variables was evaluated as low (< 0.37) using
160 concurvity measures (Amodio et al. 2014) calculated by the *mgcv*-package. Due to the

161 overrepresentation of zeros in the data (overdispersion) for the MP burden, the model was run
162 using zero-inflated Poisson error structures. Model performance was assessed using residual plots.
163 Differences in the MP burden between the basins were tested using Permanova with *station* nested
164 within *basin* as a random factor (Anderson 2001). The significance level was set at $\alpha = 0.05$; all
165 statistical analyses were conducted in R 3.5.0 (R Core Team 2014).

166

167 *Relationships between HOCs and ingested microplastic*

168 Maximum-likelihood Factor Analysis with Varimax rotation was used to assess the degree of
169 association between the chemical variables and weight-specific MP burden in the GIT. Prior to the
170 analysis, Bartlett's test of sphericity was performed to confirm patterned relationships between the
171 variables and was statistically significant ($\chi^2_{15} = 175$, $p < 0.0001$). A scree plot was used to
172 determine the number of factors to retain, and factor loadings > 0.7 were considered statistically
173 significant (MacCallum et al. 2001). When measured values were below the limit of quantification
174 (LOQ), they were imputed by LOQ divided by the square root of two (Succop et al. 2004). The
175 analyzed chemical concentrations were summed and grouped into their respective contaminant
176 groups (PCBs, PBDEs and organochlorine pesticides).

177

178 *Modeling plastic ingestion by herring*

179 To evaluate whether the observed MP burden could be predicted using ambient MP abundance
180 data and food processing rates, we modeled the ingestion of MP using literature-derived
181 parameters on food uptake, egestion, and MP abundance in the study area. The rationale is that
182 observed MP abundance in the gut would reflect average exposure levels assuming that (1) MP
183 concentrations are fairly homogeneous in the outer coastal areas (Gorokhova 2015, Gewert et al.
184 2017), which are the main feeding grounds of herring (Flinkman et al. 1998), (2) the MP abundance
185 in the water column, where the fish feed, is similar to that at the surface, where the data on the
186 relevant size fraction of MP (1- 5 mm) were collected; (3) MP ingestion by herring is non-selective
187 and thus proportional to the MP abundance in the water, and (4) gut evacuation rates are non-
188 discriminatory, i.e., MP are egested at the same rate as prey remains. Then, the MP burden (MP
189 ind^{-1}) at any given time, t , can be written as the mass balance between the uptake and loss rates
190 (Eq. 1):

191

$$192 \quad MP_t = MP(t - dt) + (IR - Eg) dt, \quad (1)$$

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

$$195 \quad IR = CMP \times CR \quad (2)$$

196 and

$$197 \quad ER = GER \times MP_t, \quad (3)$$

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

201
202 We used literature data to parameterize the model (Fig. S1, Table S2). The MP concentrations in
203 the target size range (1-5 mm) from surface waters in the outer Stockholm archipelago (Gewert
204 et al. 2017) were used as CMP values. Clearance rates were estimated using reported feeding
205 rates for North Sea herring on *Calanus finmarchicus*, a copepod of similar size as the
206 microplastics considered here, and the main prey for herring (Varpe and Fiksen 2010) (see
207 Supporting Information 1.1 for derivation of CR). As published gut evacuation rates for adult
208 herring were not available, we used experimental values reported for other clupeids of similar
209 size, European pilchard (*Sardina pilchardus*) (Costalago and Palomera 2014) and South
210 American pilchard (*Sardinops sagax*) (van der Lingen 1998), which have similar feeding
211 ecology and physiology as Baltic herring (Collard et al. 2017). The physiological rates used in
212 the model corresponded to the average size of our fish.

213
214 The model was implemented using STELLA® ver. 9.4.1 software (iSee systems, Inc. Lebanon,
215 NH, U.S.A.) to estimate MP burden ($MP\ ind^{-1}$) dynamics in a fish population at a given MP
216 abundance. The intrapopulation variability was simulated using a Monte Carlo generator with
217 1000 permutations (details on the simulation settings are provided in Supporting information 1.2).
218 To validate the model, we compared the simulated data distribution from the model to the field
219 data using descriptive statistics, χ^2 and the two-sample Cramér-von Mises tests.

220

221 **Results**

222 *Observed MP burden*

223 Particles identified by visual inspection as MP were found in 44 out of the 130 individuals (33.8%;
224 range: 0 to 51 pieces of plastic fiber and/or fragments ind^{-1}). In those 44 individuals, the mean
225 abundance was 7.8 ± 12.2 particles ind^{-1} (\pm SD). The dominant type of MP were fibers of various

226 colors (87.6%), while fragments were less frequent (12.4%). However, the black fibers were
227 identified as non-plastic by the hot needle test and were, therefore, excluded from further analyses.

228 When the black fibers were omitted, only 37 individuals contained MP (28.4 %; range: 0 to 51
229 MP) with a mean abundance of $8.4 \text{ MP ind}^{-1} \pm 12.8$. The proportion of the fibers was 86.2%. When
230 all examined individual were considered, the population average was 2.4 MP ind^{-1} with the 95%
231 bootstrap confidence interval ranging 1.4 - 4.2 MP ind^{-1} . The variation in the MP burden between
232 the stations and basins was high (Fig. 2, Supporting Information Table S1), and we did not find
233 any significant differences in the MP burden between the basins (*station* nested within *basin* as a
234 random factor, pseudo $F_{4,117} = 1.1$, $p = 0.37$).

235

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

237 The model predicted that 81% of fish contained MP, with a mean MP burden of 4.7 MP ind^{-1} ;
238 these values were about twice as high as the observed values. The ranges of the frequency
239 distributions for the simulated and observed values were overlapping, although the field
240 observations were more strongly skewed towards zero values compared to the model prediction
241 (Fig. 3 A, Supporting Information Table S3). The difference between the distributions was
242 statistically significant (Cramér-von Mises $T = 144$, $p < 0.0001$). When zeros were excluded, the
243 distributions, albeit still significantly different (Cramér-von Mises $T = 23.4$, $p < 0.0001$), became
244 more similar (Fig. 3 B, Supporting Information Table S 3), indicating that much of the difference
245 between the distributions was driven by the significantly higher proportion of zero observations
246 in the field data ($\chi^2 = 162.3$, $p < 0.0001$).

247

248 *Linkage between MP intake and HOCs*

249 We found no relationship between the weight-specific MP burden and the concentration of any of
250 the HOCs (Fig. 4). As a variable, weight-specific MP burden loaded weakly and negatively (-0.13)
251 on the first axis and moderately positive (0.62) on the second axis. In contrast, the organochlorine
252 pesticides and PBDEs loaded significantly and positively on the first axis, while the PCBs loaded
253 moderately positive (0.55) on the first and significantly positive (0.83) on the second axis.
254 Together, the two factors explained a cumulative variance of 85.1% (Supporting Information Table
255 S4). Hence, no contaminant group had loadings clustering with those for the weight-specific MP
256 burden.

257

258 *Biological factors related to MP burden*

259 The MP burden was positively and nearly linearly related to fish *body weight* (GAM, $\chi^2 = 26.5$, p
260 < 0.0001 , Fig. 5 A). In contrast, a negative effect was found for *reproductive phase*, where MP
261 burden was significantly lower in fish that had reached sexual maturity (GAM $\chi^2 = 56.4$, p
262 < 0.0001 , Fig. 5 B). *Gut fullness* only had a negative effect on MP burden when the GIT was empty
263 of food items (GAM $\chi^2 = 53.6$, $p < 0.0001$, Fig. 5 C) while *Age* displayed a weak negative
264 relationship with MP burden (GAM $\chi^2 = 6.8$, $p < 0.01$, Fig. 5 D). However, albeit statistically
265 significant, the effect of *Age* was not particularly strong and most probably of low biological
266 importance.

267

268 **Discussion**

269 *Microplastics are not very common in herring guts*

270 Microplastics (mostly fibers) were found in about one third of the fish. While these values are in
271 good agreement with those reported for herring by Beer et al. (2018) for the central Baltic Sea
272 (20% containing MP, with 93% fibers), other studies found considerably lower MP frequency of
273 occurrence and fiber contribution to total MP in herring. Both Foekema et al. (2013) and Rummel
274 et al. (2016) found plastics in only 2% of herring samples from the North Sea and the Southern
275 Baltic Sea, with fibers accounting for less than 10% of MP. Having excluded fibers from their
276 analyses, Budimir et al. (2018) reported a frequency of occurrence as low as 1.8% in herring from
277 the northern Baltic Sea. These discrepancies between different studies could be related to
278 differences in fish size and gut fullness. For example, Foekema et al. (2013) used fish that were
279 considerably larger (>200 mm total length) which most likely already had switched from filter
280 feeding to raptorial feeding on larger prey (Huse and Toresen 1996). This change in feeding mode
281 would result in a lower ingestion rate of zooplankton-sized plastic particles and thus in a lower
282 overall MP burden. In the study of Rummel et al. (2016), many fish stomachs were empty, which
283 probably was related to arrested feeding in concert with spawning, and, possibly, stress-induced
284 gut evacuation caused by the fish sampling (Wilkins 1967, Vinson and Angradi 2011). This lends
285 further support to our findings that MP burden increases with fish size (cf. Beer et al. 2018) and
286 decreases with reproductive phase. In addition, one would expect the amount of ingested MP to
287 scale with the absolute size of gut bolus or gut fullness. However, since this relationship was weak

288 (Fig. 5 C), our findings only partly support this expectation. One possible explanation for this
289 could be slower egestion of MP compared to prey, similar to the selective retention of plastic fibers
290 in amphipods (Au et al. 2015) and fragments in cladocerans (Ogonowski et al. 2016), which would
291 result in a temporary accumulation of MP in the fish gut and obscure the expected positive
292 relationship between the gut fullness and MP burden. While fish size appears to be the strongest
293 covariable for standardizing internalized MP content, gut fullness was also influential, particularly
294 for fish with no food in the GIT, which may occur during periodic fasting (Darbyson et al. 2003).

295
296 The range of the MP burden predicted by our simple model was similar to that observed in the
297 field caught specimens, although the proportion of fish predicted to contain MP was more than
298 two-fold higher. This is, however, not surprising because, frequency of zero values was driven by
299 the CMP variability that was derived from surface collection of MP. However, contrary to the
300 model assumption of homogeneous MP distribution in the water column, it is likely that the MP
301 distribution is patchy, their concentrations vary with depth (Gorokhova 2015) and form aggregates
302 too large to be mistaken for food (Long et al. 2015, Lagarde et al. 2016). Therefore, the distribution
303 of MP concentrations originating from surface collections and used to model MP encounter rate
304 might not reflect the actual situation. The observed population MP burden was also more variable,
305 which is likely to be related to diel variations in feeding and gut evacuation under natural
306 conditions (Seyhan and Grove 2003) not accounted for by the model. Other biological factors,
307 such as maturity level, ontogenetic changes in feeding, and behavior, also may have affected the
308 probability of MP ingestion and thus contributed to intrapopulation variability in the MP burden.
309 Finally, fishing methods (which may induce gut evacuation) and time of capture (which may
310 reflect diurnal differences in feeding activity) may have contributed to the observed discrepancy
311 in the MP burden distribution. Nevertheless, given the simplicity of the model and the uncertainties
312 associated with its parameters, the predicted values were sufficiently close to those found in the
313 field, indicating that MP uptake can be predicted provided that we have reliable MP abundance
314 estimates.

315

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

317 The transfer of hydrophobic contaminants from ingested plastics to biota has been described as the
318 so-called "Trojan horse" effect (Cole et al. 2011). While this transfer has been demonstrated under
319 laboratory conditions (Besseling et al. 2013, Rochman et al. 2013, Browne et al. 2013, Batel et al.
320 2016), recent modelling studies indicate that natural sources play a much more important role than

321 MP in explaining HOC bioaccumulation patterns in aquatic organisms (Koelmans 2015, Koelmans
322 et al. 2016). We did not find any correlation between HOC concentrations in herring muscle and
323 MP burden, although it could be argued that omitting small MP (< 1 mm) from our analysis, could
324 have biased the results. Indeed, by focusing on the larger MP, we ignored potentially important
325 influence of a higher total surface area and thus higher HOC desorption rates (Hendriks et al. 2001,
326 Hartmann et al. 2017). However, the ingestion of such small particles by fish of this size is rather
327 unlikely, because filter-feeding herring have a relatively low capacity to retain small particles due
328 to their rather wide gill raker spacing (Gibson 1988, Collard et al. 2017) and actively avoid smaller
329 prey while feeding raptorially (Aro et al. 1989, Casini et al. 2004). In fact, this line of reasoning
330 has been supported by several other studies reporting a predominant retention of MP of > 1 mm
331 by similarly-sized herring (Lenz et al. 2016, Collard et al. 2017, Beer et al. 2018). Moreover, given
332 the short residence time (Grigorakis et al. 2017) of internalized plastics and the slow desorption
333 kinetics of many HOCs, the lack of correlation between the MP and organic contaminants is rather
334 expected and in line with other reports for fish and other aquatic animals (Herzke et al. 2016, Rehse
335 et al. 2018, Kleinteich et al. 2018).

336
337 Causality is difficult to prove using environmental samples, where many different parameters may
338 affect contaminant body burden of an organism (Hartmann et al. 2017), including various biotic
339 factors that have significant effects on both MP (this study) and HOC levels (Persson et al. 2013,
340 Silva Barni et al. 2014). However, our findings suggest that there is no tenable relationship
341 between the MP intake and tissue contaminant concentrations in the Baltic herring (Fig. 4).
342 Similarly, no correlation has been found between the amount of ingested plastic and HOC
343 concentrations in northern fulmars (*Fulmarus glacialis*) from the Norwegian coast (Herzke et al.
344 2016), even though the birds had ingested much larger amounts of plastic and their gut passage
345 time for plastic debris is several orders of magnitude longer than in herring (Ryan 2015). This lack
346 of relationship is also supported by the relatively constant MP burden observed in Baltic herring
347 over the past three decades (Beer et al. 2018), whereas muscle concentrations of HOCs decreased
348 significantly (Bignert et al. 2016). The mass-balance model indicates that our measurements of the
349 MP burden are ecologically plausible given the currently reported abundances of MP in the Baltic
350 surface water, thus supporting the reliability of the MP burden estimates in the Baltic herring
351 reported here and in other studies and providing confidence in the methods employed. Taken
352 together, these findings contrast the currently held paradigm that microplastics are an important
353 source of HOCs in aquatic organisms (Mato et al. 2001, Rochman et al. 2013).

354

355 *Conclusions*

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

363

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376

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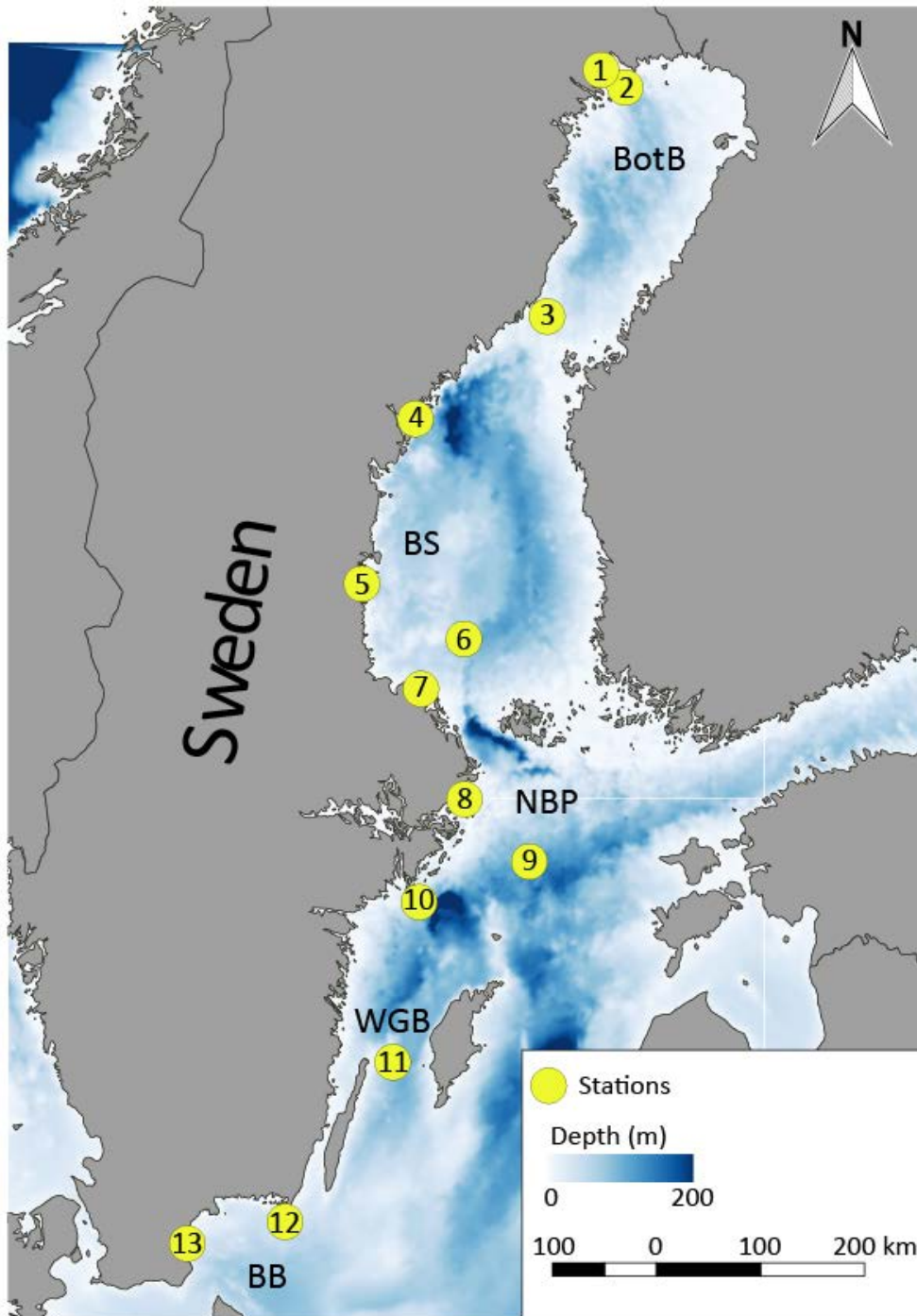
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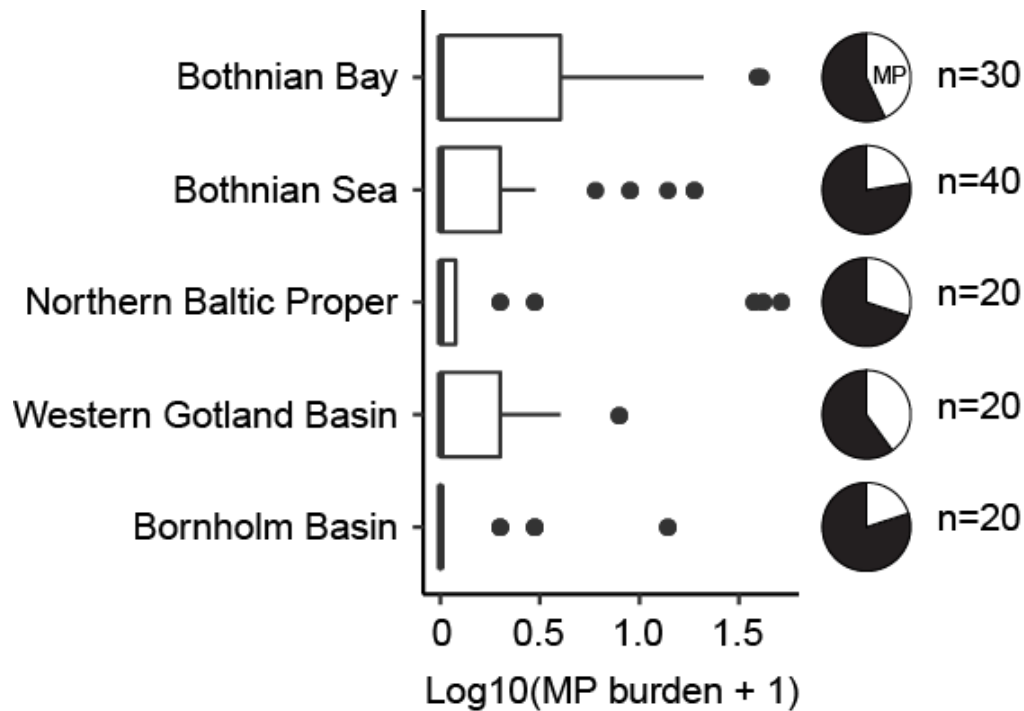
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555 **Figures and tables**



556
557 **Figure 1.** Sampling sites within the Swedish National Monitoring Program for Contaminants in
558 Marine Biota included in this study, BotB Bothnian Bay, BS Bothnian Sea, NBP Northern Baltic
559 Proper, WGB Western Gotland Basin and BB Bornholm Basin. 1 Rånefjärden, 2 Harufjärden, 3
560 Holmöarna, 4 Gaviksfjärden, 5 Långvindsfjärden, 6 Bothnian Sea offshore site, 7
561 Ängsskärsklubb, 8 Lagnö, 9 Baltic proper offshore site, 10 Landsort, 11 Byxelkrok, 12 Utlängan
562 and 13 Western Hanö bight.



563
564 **Figure 2.** Boxplot of Log10-transformed MP abundance in the gastrointestinal tract (GIT) of
565 herring per basin ordered from north to south. Data are presented as medians (vertical lines), inter
566 quartile range, IQR (boxes), 1.5 IQR (whiskers) and outliers (points) being > 1.5 IQR. The black
567 slices of the pie charts indicate the proportion of examined herring with no MP in the GIT.

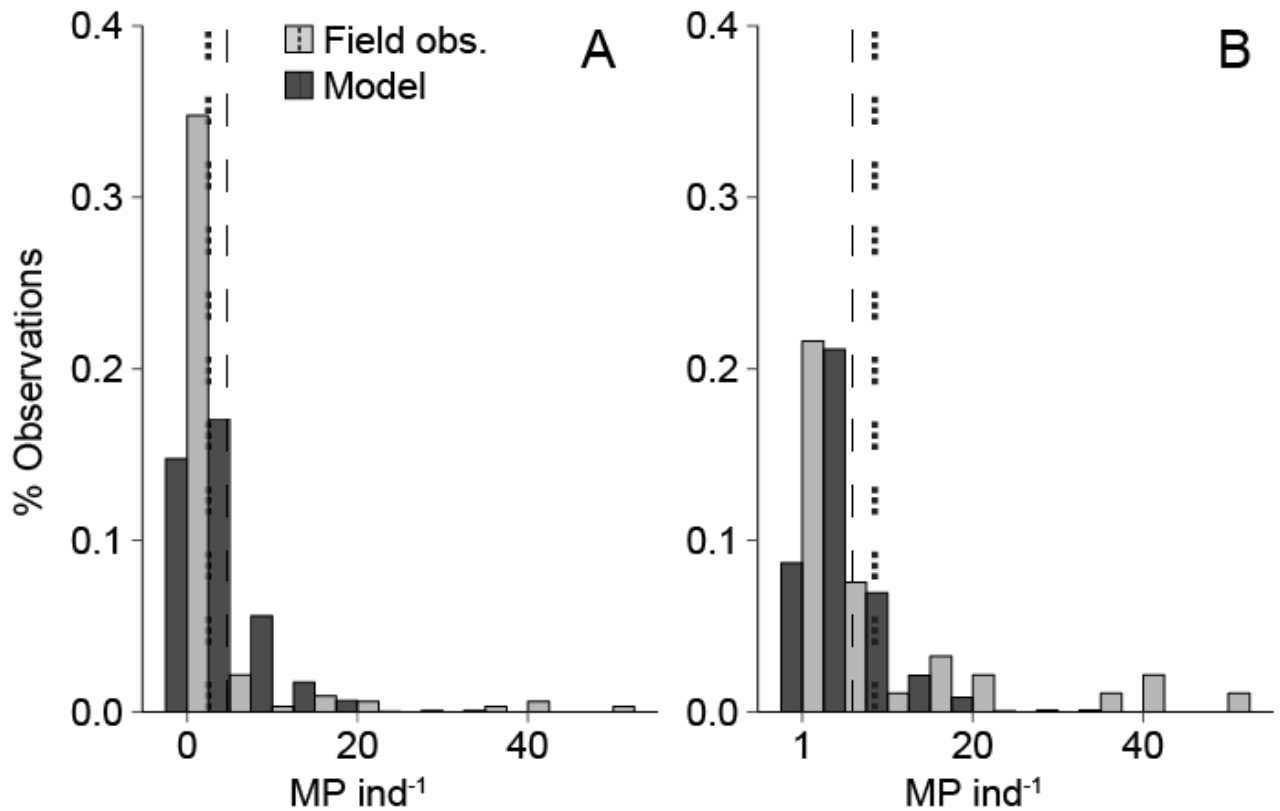


Figure 3. Frequency distribution of the MP burden based on the model simulations (dark grey bars) and field observations (light grey bars). Panel A shows the entire dataset and panel B presents only fish with MP in the GIT (i.e., the non-zero values). The dashed vertical lines indicate the mean values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

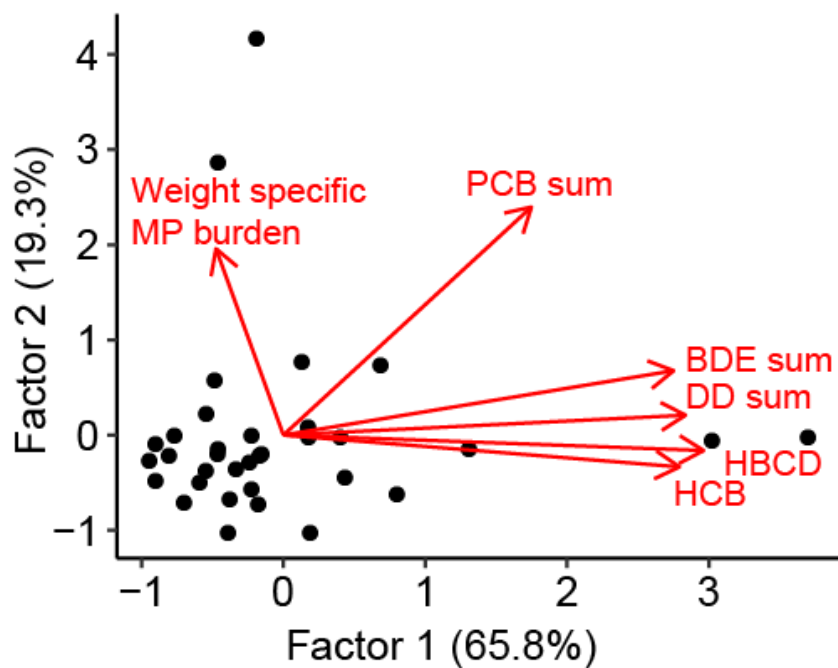


Figure 4. Factor scores (axes) and loadings (arrows) of contaminants (HBCD, HCB and the sum of PCBs, BDEs and DDs) and weight-specific MP burden.

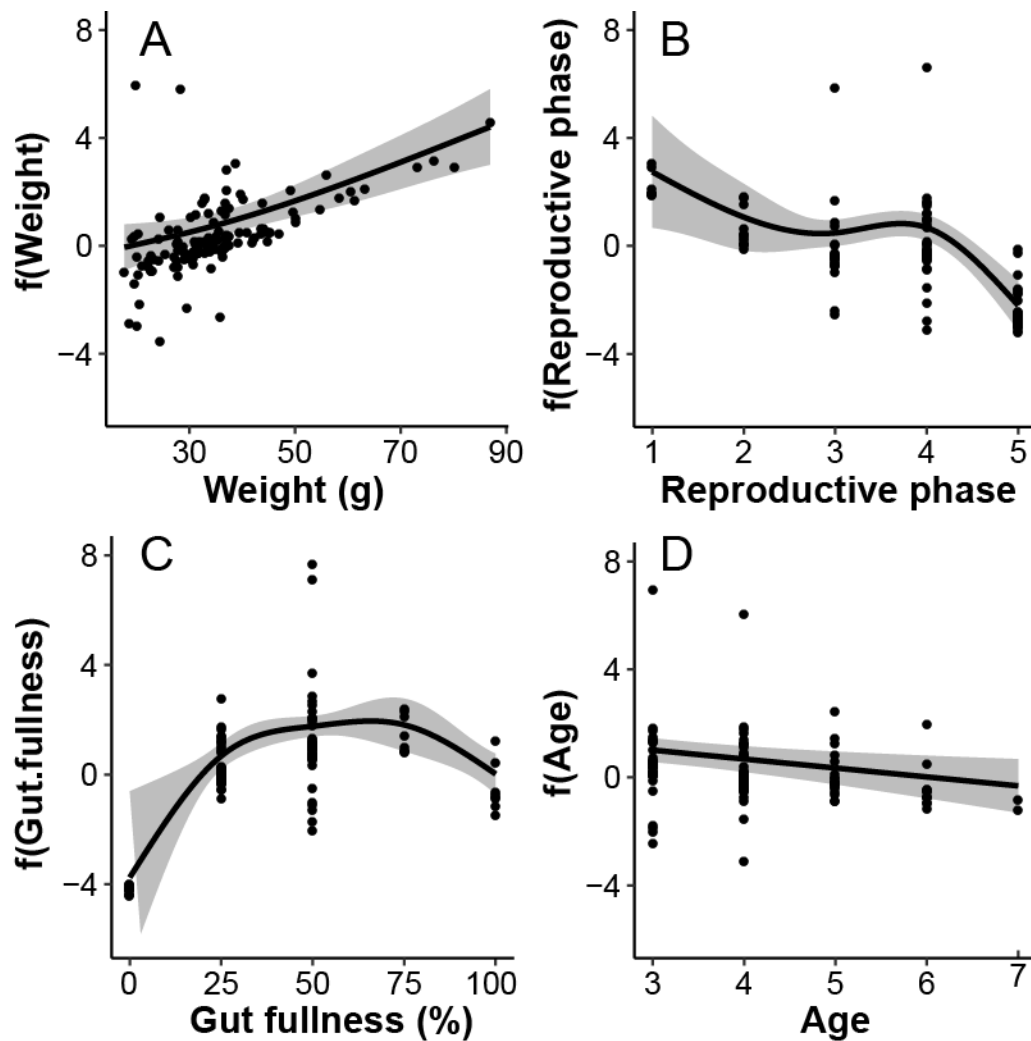


Figure 5. Generalized additive models (GAMs) showing partial response curves for the explanatory biological variables: *body weight* (A), *reproductive phase* (B), *gut fullness* (C) and *age* (D). The classes for *reproductive phase* correspond to: 1 = deformed gonads, 2 = post spawned, 3 = juvenile,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

