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1 **TRACE METAL ELEMENTS AND ORGANIC CONTAMINANTS ARE DIFFERENTLY**
2 **RELATED TO THE GROWTH AND BODY CONDITION OF WILD EUROPEAN SEA BASS**
3 **JUVENILES**

4

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27 **ABSTRACT**

28 Chemical contaminants are one of the causes of the ongoing degradation of coastal and
29 estuarine nurseries, key functional habitats in which the juveniles of many marine species
30 grow. As chemical contaminants can cause a decrease in the energy available and induce
31 defence mechanisms reducing the amount of energy allocated to life history traits,
32 quantifying their effect on the fitness of juvenile fish is key to understand their population-
33 level consequences. However, these effects are primarily estimated experimentally or in the
34 wild but on a limited number of contaminants or congeners that do not reflect the wide variety
35 of chemical contaminants to which juvenile fish are exposed. To address this issue, we
36 measured concentrations of 14 trace metal elements (TMEs) and bioaccumulative organic
37 contaminants (OCs) in European sea bass juveniles (1-year-old) from three major French
38 nurseries (Seine, Loire and Gironde estuaries). We tested the hypotheses that (i) levels and
39 profiles of contaminants differed among studied nurseries, and ii) fish growth and body
40 condition (based on morphometric measurements and muscle C:N ratio) were lower in
41 individuals with higher contaminant concentrations. Multivariate analyses showed that each
42 nursery had distinct contaminant profiles for both TMEs and OCs, confirming the specific
43 contamination of each estuary, and the large array of contaminants accumulated by sea bass
44 juveniles. Increasing concentrations in some TMEs were associated to decreased growth,
45 and TMEs were consistently related to lower fish body condition. The effect of OCs was more
46 difficult to pinpoint possibly due to operational constraints (i.e., analyses on pooled fish) with
47 contrasting results (i.e., higher growth and decreased body condition). Overall, this study
48 shows that chemical contaminants are related to lower fish growth and body condition at an
49 early life stage in the wild, an effect that can have major consequences if sustained in
50 subsequent ages and associated with a decline in survival and/or reproductive success.

51 Keywords: Chemical contaminants, *Dicentrarchus labrax*, Early-life stages, Inorganic
52 elements, Anthropogenic impacts, Marine pollution

53 INTRODUCTION

54 Coastal and estuarine areas play a central role in the dynamics of exploited marine
55 fish populations as they hold nurseries of many species (Beck et al., 2001; Day et al., 2013).
56 These ecosystems have particularly rich benthic communities that favour the growth and
57 survival of juvenile fish, but are also characterised by highly variable physico-chemical
58 characteristics (Day et al., 2013). Although species inhabiting nurseries are adapted to such
59 environmental variations (Elliott and Quintino, 2007), additional stress factors induced by
60 human activities can set strong constraints on juveniles living in nurseries (e.g. Courrat et al.,
61 2009; Hughes et al., 2015; Vasconcelos et al., 2007). Indeed, about 23% of the world's
62 human population lives within 100 km of coastline (IPCC, 2007) and such dense
63 industrial/agricultural/urban areas are major sources of chemical contaminants (CCs),
64 reaching estuaries through tributaries, direct discharge of effluents, runoff or atmospheric
65 transport (e.g. Dendievel et al., 2020; Johansson et al., 2019; Pacyna and Pacyna, 2001).
66 Understanding the impact of chemical contaminants on the early life history of fish is
67 therefore required to understand and predict variations in the nursery function of estuaries
68 and coastal areas.

69 Chemical contaminants include both trace metal elements (TMEs) and organic
70 contaminants (OCs) and have long been identified as threats to marine biodiversity (CBD,
71 2010). TMEs are emitted into the environment by natural sources (e.g. volcanism) and/or
72 human activities, and can be categorised into essential TMEs (E-TMEs) with known
73 biological functions, and non-essential TMEs (NE-TMEs; Mason, 2013). E-TMEs are
74 detrimental at low and high concentrations (characterising deficiencies and toxicities), while
75 NE-TMEs can be toxic even at low concentrations (Mason, 2013). OCs are synthetic
76 compounds used in industrial, agricultural and domestic contexts (Jones and Voogt, 1999);
77 they include for instance polychlorinated biphenyls (PCBs), organochlorine pesticides
78 (OCPs, such as DDT or dieldrin), and perfluoroalkyl substances (PFASs). Juvenile fish living
79 in estuaries are therefore potentially exposed to a wide variety of OCs (Munschy et al., 2011;

80 Williams and McCrary, 2021) that can have deleterious effects as previously shown under
81 experimental conditions (e.g. Foekema et al., 2014; Horri et al., 2018; Ankley et al., 2021).
82 Although such controlled experiments are required to pinpoint the effect of contaminants on
83 fish physiology and fitness, their implication for natural populations is difficult to ascertain as
84 experiments usually focus on a limited number of contaminants or congeners. Furthermore,
85 the acute exposures to contaminant generally used in experiments are unlikely to occur in
86 the wild because of the large spatio-temporal variations in contaminant concentrations (e.g.
87 Dendievel et al. 2020), and because fish can avoid areas with highly concentrated
88 contaminants (Tierney, 2016). Because their continuous input, persistence, and increasing
89 diversity can directly impact individuals' fitness, there is therefore a clear need for studies
90 quantifying the effects of a wide variety of chemical contaminants on juvenile fish living in
91 nurseries *in situ*.

92 Field studies have used chemical contaminants to characterise estuary 'quality' (e.g.
93 Couderc et al., 2015; Courrat et al., 2009) and their effect on the phenotype of individuals
94 (e.g. Li et al., 2010). These studies focussed primarily on the effect of contaminant
95 concentrations on fish growth because of its direct relationship with individuals' survival rates
96 (Cushing, 1975), and future reproductive investment (Hixon et al., 2014). Fish mortality is
97 also challenging to measure accurately in the wild and reproduction is not a relevant life
98 history trait in juveniles. To complement growth measurements, field studies also commonly
99 use morphometric measures of body condition, based on fish length and weight (Froese,
100 2006), under the assumption that the body weight for a given length reflects individuals'
101 energy reserves (Peig and Green, 2010). Therefore, the allocation of energy to defence
102 mechanisms against chemical contaminants can lead to a decrease in body condition and
103 growth (e.g. Snyder et al. 2019; Petitjean et al. 2020). However, many field studies focused
104 on a limited set of contaminants (e.g. Couderc et al. 2015), sometimes relied on
105 measurements of contaminants in food (e.g. Gilliers et al., 2006), and emerging
106 contaminants such as PFASs are often ignored (Ankley et al., 2021). Furthermore, OCs such
107 as PCBs and DDTs may have obesogenic effects leading to an increase in body weight

108 (Lyche et al., 2010), which may complicate our overall understanding of the impact of
109 chemical contaminations. Addressing these limitations requires quantifying the
110 concentrations of chemical contaminants in the most complete way as possible to
111 characterise their overall impact on fish growth and body condition in natural populations.

112 In this study, we tested the hypotheses that levels and profiles of TMEs and
113 persistent and bioaccumulative OCs were nursery-specific and that fish growth and body
114 condition were lower in individuals with higher contaminant concentrations. To this end, we
115 used data collected in sea bass juveniles (*Dicentrarchus labrax*) aged 1; a commercially
116 important demersal species with declining stocks in Western Europe following overfishing
117 and low recruitment rates (ICES, 2020). A survey was therefore set up to quantify the
118 abundance of juvenile sea bass in three major sea bass nurseries along the French western
119 coasts that receive inputs from major urban, industrial and agricultural anthropogenic
120 activities. We used fish sampled during this survey over two consecutive years in which we
121 measured whole-body concentrations of 9 E-TMEs, 5 NE-TMEs and 3 families of OCs that
122 are representative of various anthropogenic sources and globally distributed (Dachs et al.,
123 2002; Johansson et al., 2019; Pacyna and Pacyna, 2001; Sánchez-Quiles et al., 2017). We
124 first determined the degree to which each CC differed among nurseries and among years.
125 We then tested whether there was a relationship between the selected CCs and the growth
126 and body condition of sea bass juveniles using two separate multivariate analyses (one
127 conducted only on TMEs at the individual level, and another one focussed on anthropogenic
128 OCs and NE-TMEs).

129

130 **MATERIAL AND METHODS**

131 1- Studied nurseries and sample collection

132 European sea bass juveniles were collected during the survey NOURDEM (Drogou et al.,
133 2019) in Gironde, Loire, and Seine estuaries, the largest of France's western coast, opening
134 to the Bay of Biscay and the English Channel (Supp. Fig. 1). The survey took place every

135 year in July (Loire), August (Seine), and September (Gironde), with dates varying slightly (2-
136 5 days) to minimize tidal currents and changes in upstream salinity limits. In each estuary,
137 *ca.* 70 tows were conducted onboard small local professional trawlers (*ca.* 10 m long;
138 draughts < 2 m) to enable the sampling of foreshore areas at mid-tides (Le Goff et al., 2017).
139 Tows lasted 15 minutes with a traction speed set at 3.5 knots and the bottom otter-trawl (7 m
140 wide, 2.40 m high) was specifically designed to capture demersal fish juveniles (Le Goff et
141 al., 2017). Overall, the sampling area covered the estuaries from upstream salinity limits
142 down to their mouth (*ca.* Gironde: 863 km², Loire: 140 km², Seine: 193 km²). After each tow,
143 the whole catch was sorted and sea bass with length consistent with known distribution of
144 age-1 individuals were euthanized by placing in a tray with a mixture of cold water and ice
145 and stored frozen individually until further treatment in the laboratory (injuries resulting from
146 capture are rare for sea bass juveniles and all other sea bass juveniles were subsequently
147 released; Le Goff et al. 2017). We collected a total of 105 fish for this study: 30 fish in Loire
148 and Seine (2018), and 15 fish in Gironde, Loire and Seine (2019).

149

150 2- Sample preparation

151 All sampling equipment and utensils were cleaned rigorously and adapted to meet the
152 requirements of the different contaminants. Stainless steel dissecting forceps, scalpels and
153 blades were thoroughly rinsed with methanol and ultra-pure water between each sample,
154 while acid-cleaned glassware oven-baked at 450 °C for 8 hours was used to store the
155 samples at each step of fish preparation. All sample preparation steps were also performed
156 in positive pressure laboratories. We first defrosted fish at ambient temperature and rinsed
157 them individually with ultra-pure water to reduce the risk of external contamination. We
158 measured fish total length (nearest 0.5 cm), weight (nearest mg), and took a few scales to
159 confirm the age of each fish (based on growth rings). We then collected a small piece of
160 white muscle dorsally (< 3% total weight) for carbon and nitrogen measurements (C:N
161 ratios). The digestive tracts were subsequently emptied and ground with the remaining body

162 in a glass blender with stainless steel blades. After freeze-drying, the samples were further
163 ground with a ball mill MM400 (Retsch) using zirconium oxide bowls and marbles. All fish
164 were processed individually for TME analyses while pools of five individuals within the same
165 trawl were processed for OC analyses (size differences within trawls were minimal).

166

167 3- Growth and body condition indices

168 As all sampled fish were 1-year-old, differences in their length reflected differences in growth.
169 There were slight initial differences in juvenile length among sites, fish being slightly longer in
170 Loire compared with Seine and Gironde estuaries (mean \pm SD; Gironde: 15.50 ± 1.57 ; Loire
171 16.51 ± 0.94 ; Seine 15.41 ± 0.97 cm; ANOVA: $F_{2,102} = 13.30$, $P < 0.001$). To estimate fish
172 body condition, we used a morphometric parameter (the Scaled-Mass Index \widehat{M}_i ; Peig and
173 Green, 2009) and a biochemical parameter (the C:N ratio). \widehat{M}_i is a morphometric condition
174 index parameterised using individuals' length (L_i) and body weight (M_i) and more weakly
175 affected by differences in body size than other morphometric indices (Peig and Green, 2010).

176 \widehat{M}_i is calculated as: $\widehat{M}_i = M_i \left(\frac{L_0}{L_i}\right)^{b_{SMA}}$, with b_{SMA} the scaling exponent of the weight-length
177 relationship estimated by a standardized major axis (SMA) regression between the
178 logarithms of body weight and size, and L_0 a reference size (i.e. the arithmetic mean length
179 calculated across all sampled individuals). The C:N ratio is also a proxy of body condition,
180 reflecting the lipid content of tissues (Hoffman et al., 2015; Post et al., 2007). The portion of
181 muscle samples collected for C and N analyses was weighed (0.40 ± 0.05 mg dry weight)
182 and C and N contents were measured using a Thermo Scientific Flash EA1112 elemental
183 analyser.

184

185 4- Trace metal elements analyses

186 We used aliquots of whole fish homogenised powder (50 ± 10 mg) to measure total mercury
187 (Hg) concentrations by atomic absorption spectrophotometry (Advanced Mercury Analyser,

188 ALTEC AMA-254). Measurements were carried out by strictly following the standard
189 operating procedure described in US-EPA method 7473 (U.S. Environmental Protection
190 Agency, 1998). We then measured concentrations of E-TMEs (arsenic (As), cobalt (Co),
191 chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), vanadium (V),
192 and zinc (Zn)) and NE-TMEs (silver (Ag), cadmium (Cd), mercury (Hg), lead (Pb), and 14
193 elements of the Rare Earth Elements (REE) family (lanthanum (La), cerium (Ce),
194 praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd),
195 terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb),
196 lutetium (Lu)). Aliquots (*ca.* 200 mg of homogenised powder) were placed in Teflon bombs
197 and mineralized with ultra-pure HNO₃ acid and water using a microwave system (ETHOS-
198 UP, Milestone). Finally, digests were diluted to 50 mL with ultra-pure water and the
199 concentration of TMEs were quantified by inductively coupled plasma mass spectrometry
200 (ICP-MS, ICAP-Qc ThermoFisher). The quality assurance of all TME analyses relied on
201 blanks, internal standard controls and on the accuracy and reproducibility of data relative to
202 certified reference materials (CRM). Blank values were systematically below detection limits
203 and CRM values concurred with certified concentrations. The CRM used were IAEA-407 (fish
204 homogenate, International Atomic Energy Agency/IAEA) and IAEA-142 (mussel
205 homogenate, IAEA) for Hg; IAEA-407, DORM-4 (fish protein, National Research Council of
206 Canada/NRCC) and DOLT-5 (dogfish liver, NRCC) for other TMEs; BCR-668 for REE
207 (mussel tissue, Joint Research Centre of the European Commission). Detection limits and
208 average recovery rates are detailed in Supp. Table 1. All TMEs are reported in $\mu\text{g g}^{-1}$ of dry
209 weight (dw), except REE which were reported in ng g^{-1} dw.

210

211 5- Organic contaminant analyses

212 We focussed on persistent organic pollutants of three families representing major concerns
213 for marine environments: PCBs, OCPs, and PFASs. Detailed analytical procedures for these
214 measurements can be found in Munsch et al. (2020) and references therein (Supp. Table

215 2). The analyses of PCBs and OCPs were performed using gas chromatography coupled to
216 high-resolution mass spectrometry (GC-HRMS, Hewlett-Packard 6890 gas chromatograph
217 coupled to a Micromass AutoSpec Ultima mass spectrometer), and PFASs were measured
218 using ultra-performance liquid chromatography coupled to tandem mass spectrometry
219 (UPLC-MSMS, Acquity UPLC coupled to Xevo® TQ-S micro, Waters Corp.). For each of the
220 15 pools of samples (75 individuals), we measured 18 different PCBs including the 6
221 indicator PCBs (i-PCBs) and the 12 dioxin-like PCBs (dl-PCBs; detailed in Supp. Table 3). All
222 PCBs were summed and referred to as Σ PCB hereafter. Among OCPs, we measured
223 dieldrin and 5 different DDT isomers (detailed in Supp. Table 3); these isomers were also
224 summed and are referred to Σ DDT hereafter. Among PFASs, we focussed on the C₄- to C₁₀-
225 perfluoroalkyl sulfonates (PFASs) and C₆- to C₁₄ perfluorocarboxylic acids (PFCAs). As the
226 perfluorooctane sulfonate (PFOS) accounted for over 90% of the total concentrations of
227 PFASs in all samples, we decided to focus on this compound among PFASs. Moreover, to
228 keep the number of variables low, we decided to focus on perfluorononanoic acid (PFNA; C₉
229 PFCA), and the sum of C₁₂-C₁₄ PFCAs, referred to as the very long chain PFCAs (Σ vlc-
230 PFCA) as PFNA and Σ vlc-PFCA showed peculiar profiles in our samples. We carried out all
231 OC analyses following strict QA/QC procedures, i.e. clean and low-dust atmosphere, positive
232 pressure laboratories, in-house quality control samples, procedural blanks, quantifications
233 using external calibration, addition of labelled compounds before extraction to calculate
234 recoveries, and participation in the Quasimeme interlaboratory comparison tests for the
235 marine environment with satisfactory Z-scores (i.e. between -1.2 and +0.2 for OCPs, -1.2 and
236 0.0 for PCBs and -0.9 and 0.0 for PFASs). Detailed information on QA/QC performances can
237 be found in Supp. Table 3 and Supp. Table 4. All OCs were measured in ng g⁻¹ dw.

238

239 6- Statistical analyses

240 We tested the normality of each chemical contaminant (Shapiro-Wilks test) and detected
241 three outliers for Fe, Pb, and Mo (Z-scores > 7). We replaced these values by missing values

242 to calculate summary statistics and compare the concentrations of these TMEs among years
243 and nurseries. For the multivariate analyses, we replaced these values by the mean values
244 of Fe, Pb, and Mo calculated without the outliers. We then calculated summary statistics for
245 each chemical contaminant (mean, median, and interquartile range -IQR) in each nursery
246 and year to provide descriptive data for comparative purposes. As all CCs had substantial
247 deviations from normality, we log-transformed their values to test whether there were
248 significant differences among nurseries and years in their concentrations (adjusted table-
249 wise p-value for TMEs and OCs: 0.004 and 0.010; Bonferroni correction). For these
250 analyses, we used individual data for TMEs (N = 105) and the values of OCs obtained for the
251 pools of individuals (N = 15).

252 To understand the relationship between concentrations of each contaminant, we
253 conducted two principal component analyses (PCA) based on log-transformed concentration
254 values: a first one using all measured TMEs (105 individuals, 14 variables), and another one
255 using all OCs and the sum of NE-TMEs (75 individuals, combined in 15 pools for OCs
256 measurements, 7 variables). These analyses were carried out separately to have a complete
257 investigation of TMEs (E- and NE-TMEs) at the individual level and to examine whether NE-
258 TMEs and anthropogenic OCs were negatively related to individuals' growth and body
259 condition. PCAs were implemented using the r-package 'FactoMineR' (Le et al. 2008) based
260 on centered variables. We then retained 3 principal components (PC) for the PCA loaded
261 with TMEs and and 2 PCs for the PCA loaded with OCs and NE-TMEs (see Results).

262 We quantified among-year and nursery differences in PCs using ANOVAs (for TMEs)
263 and mixed models to account for the non-independence of pools of samples measured in
264 OCs (i.e. pool identification numbers were used as a random variable). The mixed models
265 did not include interaction terms because of the limited sample size of OC measurements.
266 We tested the effect of PCs on fish body length and condition measures (\hat{M}_i and C:N ratio)
267 using linear models and linear mixed models (for PCs synthesising OCs and NE-TMEs). We
268 decided not to use PC3 of TMEs because of its positive relationship with some TMEs and
269 negative relationship with others (see Results), making its biological interpretation

270 ambiguous. The explanatory variables of full models (fixed effects) consisted in the two PCs
271 extracted from each PCA and their interactions with the sampling site ('Nursery'). We
272 included the sampling year as main effect to account for among year differences in
273 explanatory and response variables. We tested no interaction between PCs as these
274 variables are orthogonal, by definition. We estimated parameters using maximum likelihood
275 and compared the relative performance of the models based on their Akaike Information
276 Criterion for small sample size (AICc). When several models had AICc differences below
277 two, we calculated averaged coefficients with unconditional standard errors (SE) and 95%
278 confidence intervals (CI) using the r-package 'MuMIn' 1.43.17 (Barton 2020) except when the
279 best model contained none of the PCs (i.e. when PCs explained little variation in the
280 response variables). All mixed effect models were implemented in the r-package 'nlme'
281 (Pinheiro et al. 2021).

282

283 **RESULTS**

284 1- Concentrations and profiles of chemical contaminant in sea bass

285 Concentrations varied according to nurseries and/or years for all TMEs except Cr (Supp.
286 Table 5). There were significant differences among nurseries and years in As, Fe, Hg, Mn,
287 Pb, V, and Σ REE (Supp. Table 5, concentrations reported in the text are median values).
288 More specifically, concentrations in As were higher in Seine than in Loire in 2018 (4.20 vs
289 2.54 mg kg⁻¹ dw) but its overall concentration increased in 2019 (5.59 mg kg⁻¹ dw) with no
290 significant differences among nurseries (Supp. Table 5). Conversely, concentrations in Fe in
291 Loire 2019 increased compared with the previous year (from 53.6 to 61.0 mg kg⁻¹ dw) and
292 compared to other sites whose concentrations did not change (Supp. Table 5).
293 Concentrations of Hg were lower in 2018 in Loire (0.156 mg kg⁻¹ dw) but increased
294 subsequently to reach similar concentrations to those of fish sampled in Gironde and Seine
295 in 2019 (0.216 mg kg⁻¹ dw, Supp. Table 5). The concentrations of Mn were lower in Seine
296 than in Loire in 2018 (9.20 vs 12.20 mg kg⁻¹ dw), and these concentrations increased in

297 Seine in 2019 leading to no major difference among nurseries (Supp. Table 5).
298 Concentrations of Pb increased between 2018 and 2019 in Loire only (from 0.088 to 0.161
299 mg kg⁻¹ dw, Supp. Table 5). Finally, there was an increase in the concentration of V and
300 Σ REE in Loire between 2018 and 2019 (respectively from 0.19 to 0.25 mg kg⁻¹ dw and from
301 34 to 48 ng g⁻¹ dw) while these TMEs decreased in Seine. Nurseries differed significantly
302 between years for Ag, Cd, Mo, and Zn (Supp. Table 5) with particularly high levels of Ag in
303 Seine (0.115-0.240 mg kg⁻¹ dw in 2018-2019, vs 0.064 and 0.037-0.067 mg kg⁻¹ dw in
304 Gironde and Loire respectively), and Zn in Gironde (90.3 mg kg⁻¹ dw in 2019, vs 75.3-83.8
305 and 73.5-87.2 mg kg⁻¹ dw in 2018-2019 in Loire and Seine respectively), and low levels of Cd
306 in Loire (0.003-0.006 mg kg⁻¹ dw in 2018-2019, vs 0.017 and 0.008-0.013 mg kg⁻¹ dw in
307 Gironde and Seine respectively) and Mo in Gironde (0.026 mg kg⁻¹ dw in 2019, vs 0.039 mg
308 kg⁻¹ dw for both Loire and Seine in 2019). Finally, concentrations in Ag, Cd, Co, Cu, and Zn
309 were higher in 2019 than in 2018 while the concentrations in Mo were higher in 2018 than in
310 2019 (details in Supp. Table 5).

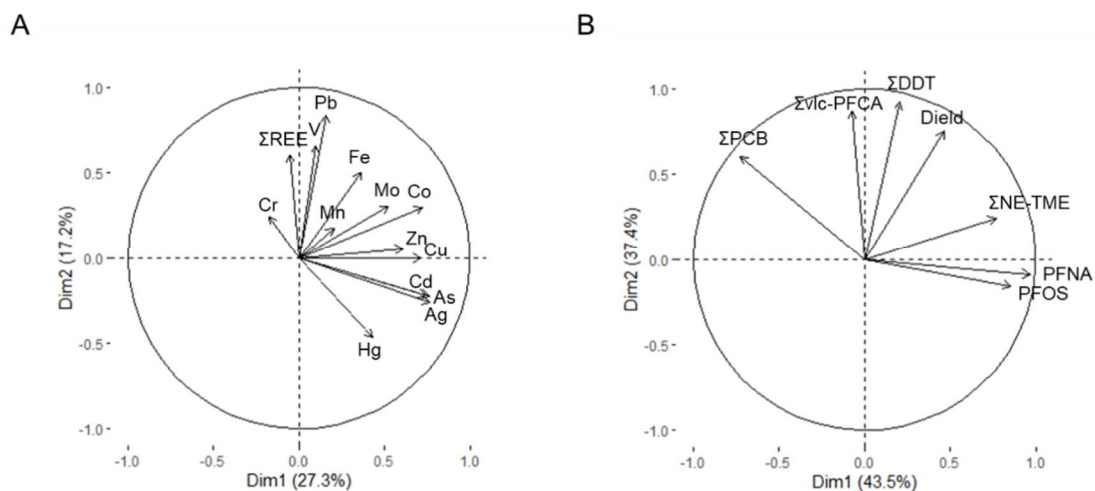
311 PCBs were by far the predominant OCs in all nurseries and years (Σ PCB > 100 ng g⁻¹
312 dw), followed by PFOS and Σ DDT (7.8-31.4 and 9.2-19.9 ng g⁻¹ dw, respectively), and
313 PFCAs and dieldrin (0.1-5.7 and 0.8-6.4 ng g⁻¹ dw, respectively). PCB contamination profiles
314 were dominated by the hexachlorinated congeners CB-153 (41%) and CB-138 (18%)
315 followed by the heptachlorinated CB-180 (14%), while dl-PCBs were 7 ± 2 times lower than i-
316 PCBs. No significant difference in Σ PCB levels was found among nurseries and between
317 years (Supp. Table 6). Σ DDT concentrations were higher in Gironde (18.4 ng g⁻¹ dw) than in
318 Seine and Loire (9.5 and 9.0 ng g⁻¹ dw, respectively) in 2019, and levels were lower in 2019
319 than in 2018 in the two latter nurseries by a factor of 1.5-2 (Supp. Table 6). The main DDT
320 isomers were *p,p'*-DDE ($80 \pm 3\%$) and *p,p'*-DDD ($15 \pm 2\%$). We measured high
321 concentrations of dieldrin in sea bass juveniles sampled in Gironde (dieldrin/ Σ DDT ratio of
322 0.38 ± 0.03) compared with fish sampled in Seine and Loire (ratio of 0.12 ± 0.02). PFOS
323 constituted 57 to 86 % of the PFASs and both PFOS and PFNA were detected in all samples
324 (Supp. Table 3). The contribution of Σ vic-PFCA to the overall concentration in PFCAs was 51

325 $\pm 2\%$, $40 \pm 4\%$, and $69 \pm 5\%$ in Gironde, Loire and Seine, respectively. The ratios of
326 Σ PFCA/PFOS clearly differed in Loire where PFOS levels were relatively higher, indicating
327 different sources of PFASs in this estuary.

328

329 2- Multivariate analyses

330 For TMEs, the first four axes of the PCA had eigenvalues over 1 but there was a clear drop
331 in the variance explained by PC4 (8%). We therefore decided to focus on the first three axes
332 that altogether explained 58% of the total variance (Supp. Table 7a). PC1 was primarily
333 related to increasing concentrations of Ag, As, Cd, Co, Cu, Mo and Zn (Supp. Table 7a; Fig.
334 1A), PC2 was related to increasing concentrations of Fe, Pb, V, and REE (Supp. Table 7a;
335 Fig. 1A) and PC3 to increasing concentrations of Cr and Mo and decreasing concentrations
336 of Mn and Zn (Supp. Table 7a). For the PCA loaded with OCs and NE-TMEs, only the first
337 two PCs had eigenvalues over 1, explaining 81% of the total variation in the loaded variables
338 (Supp. Table 7b; Fig. 1B). Increasing values of PC1 were associated with increasing
339 concentrations of PFOS, PFNA, and NE-TMEs and decreasing values of Σ PCB while PC2
340 was positively associated with OCPs, Σ PCB, and Σ vl-PFCA (Supp. Table 7a; Fig. 1B).



341

342

343 Figure 1: Projection of the different trace metal elements (TMEs; panel A) and organic contaminants
344 with non-essential metal trace elements (OCs, NE-TMEs; panel B) on the first two axes of the
345 separate principal component analyses. See Supp. Table 5 for contaminants' abbreviations.

346

347 3- Among nursery differences in overall contaminant profiles

348 There were clear differences in the PCs among nurseries and years for both TMEs and OCs
349 (Table 1; Supp. Table 8). For TMEs, PC1 values were lower in 2018 than in 2019, and PC1
350 values of fish sampled in Loire were lower than those of Seine in 2018 (Table 1; Fig. 2A).
351 Fish sampled in Seine had substantially lower PC2 values but there were clear among-year
352 differences within Loire and Seine in 2018 and 2019 (Table 1; Fig. 2B). PC3 values were low
353 in Gironde, intermediate in Loire, and high in Seine (Table 1; Fig. 2C). Differences among
354 nurseries were more pronounced in the PCA loaded with OCs and NE-TMEs: PC1 values
355 were substantially lower in fish sampled in Seine (Table 1, Fig. 2D) and PC2 values were
356 particularly high in Gironde and Seine in 2018 (Supp. Table 7; Fig. 2E).

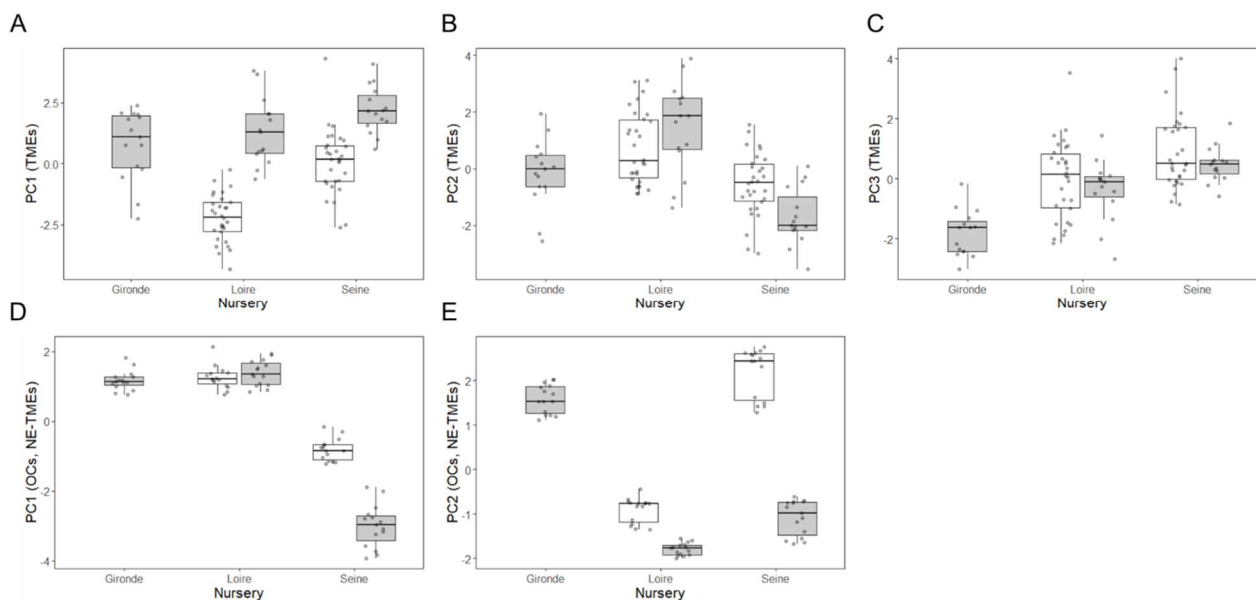
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359 Table 1: Relative performance of models testing among nursery and year differences in PCs obtained
 360 based on trace metal elements (TMEs), and non-essential TMEs and organic contaminants (OCs, NE-
 361 TMEs). Table entries: number of parameters (K), Log-likelihood (LogLik), Akaike's Information
 362 Criterion for small sample sizes (AICc), difference in AICc values relative to the best model ($\Delta AICc$),
 363 model weight (w_i). Models with $\Delta AICc \leq 2$ are presented with the first model with $\Delta AICc > 2$.
 364

Dependent variable	Model	K	LogLik	AICc	$\Delta AICc$	w_i
PC1 (TMEs)	Nursery*Year	6	-166.27	345.39	0.00	0.91
	Nursery+Year	5	-169.74	350.09	4.69	0.09
PC2 (TMEs)	Nursery*Year	6	-168.03	348.92	0.00	0.97
	Nursery	4	-174.19	356.77	7.85	0.02
PC3 (TMEs)	Nursery+Year	5	-156.54	323.68	0.00	0.48
	Nursery	4	-157.91	324.22	0.54	0.37
	Nursery*Year	6	-156.53	325.92	2.23	0.16
PC1 (OCs, NE-TMEs)	Nursery+Year	6	-44.49	102.21	0.00	0.93
	Nursery	5	-48.24	107.36	5.15	0.07
PC2 (OCs, NE-TMEs)	Nursery+Year	6	18.31	-23.38	0.00	1.00
	Nursery	5	9.67	-8.47	14.91	<0.01

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 368 Figure 2: Differences among nursery and year in the principal components extracted for trace metal
 369 elements (TMEs) alone (panels A-C) and organic contaminants with non-essential trace metal
 370 elements (OCs, NE-TMEs, panels D and E). White and grey boxes represent samples collected in
 371 2018 and 2019, respectively. Dots represent each measurement.
 372

373 4- Relationships between contaminants and fish growth and body condition

374 Juvenile sea bass growth and body condition were significantly related to their TMEs'
375 contamination (Figure 3), although this response varied depending on the considered TMEs.
376 More specifically, growth declined with increasing levels of PC1 but was unrelated to PC2,
377 which did not appear in the best models (Table 2; Fig. 3A). The effects of PC1 on fish length
378 was dependent on the nursery as the best model contained the interaction term (Table 2;
379 Fig. 3A). Fish body condition (\widehat{M}_i) declined with both PC1 and PC2 (Table 2; Fig. 3B and C).
380 These effects were consistent across nurseries and years (Table 2). Finally, the best model
381 for the C:N ratio contained none of the PCs; PC2 appeared in the second model but
382 explained little variance in C:N ratio (Supp. Table 9).

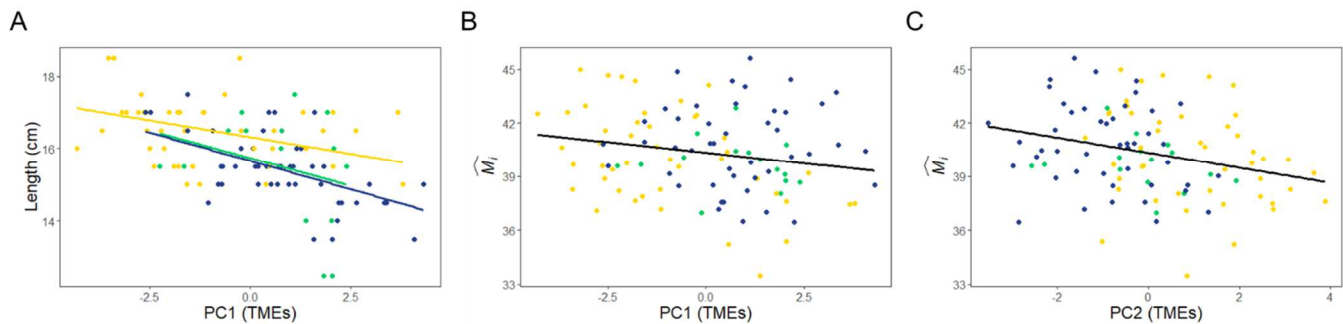
383 The effect of OCs and NE-TMEs on juveniles' growth and body condition varied
384 substantially depending on the contaminants and nurseries considered. In general, the effect
385 of OCs associated with PC2 (associated with \sum DDT, dieldrin, \sum PCB, and \sum vlc-PFCA) were
386 greater than those associated with PC1 (Table 3). There were clear single best models for
387 body length and the C:N ratio (Table 3) containing PC2 as a main effect or as an interaction
388 term with nurseries (Table 3). Overall, fish growth was positively associated with PC2 in Loire
389 and Seine but not in Gironde (Fig. 4A). For \widehat{M}_i , 5 models had Δ AICc < 2 and one of them was
390 the null model (Table 3) suggesting that the effect of OCs and NE-TMEs on fish body
391 condition were relatively weak. Averaged parameter estimates for PC1 and PC2 showed that
392 increasing concentrations of OCs and NE-TMEs led to declines in body condition (Fig. 4B
393 and C). For the C:N ratio, there was a single best model which contained only PC2 (Table 3)
394 clearly showing that there was a decline in C:N ratio increasing PC2 values (Supp. Table 10;
395 Fig. 4D).

396

397 Table 2: Relative performance of models testing the effect of principal components of essential and
 398 non-essential trace metal elements on the length, body condition index (\hat{M}_i), and C:N ratio of
 399 European sea bass juveniles. All models with $\Delta AICc < 2$ are presented along with the first model with
 400 $\Delta AICc > 2$. Table entries defined in Table 1.

Response variable	Explanatory variables	K	LogLik	AICc	$\Delta AICc$	w_i
Length	PC1*Nursery	7	-142.08	299.31	0.00	0.24
	PC1+Nursery	5	-144.50	299.61	0.31	0.21
	PC1*Nursery+Year	8	-142.06	301.61	2.30	0.08
\hat{M}_i I	PC1+PC2	4	-234.53	477.47	0.00	0.30
	PC2	3	-236.69	479.61	2.15	0.10
C:N ratio	Nursery+Year	5	259.87	-509.14	0.00	0.35
	Nursery+Year+PC2	6	260.36	-507.86	1.28	0.19
	Nursery+Year+PC1	6	259.88	-506.89	2.25	0.11

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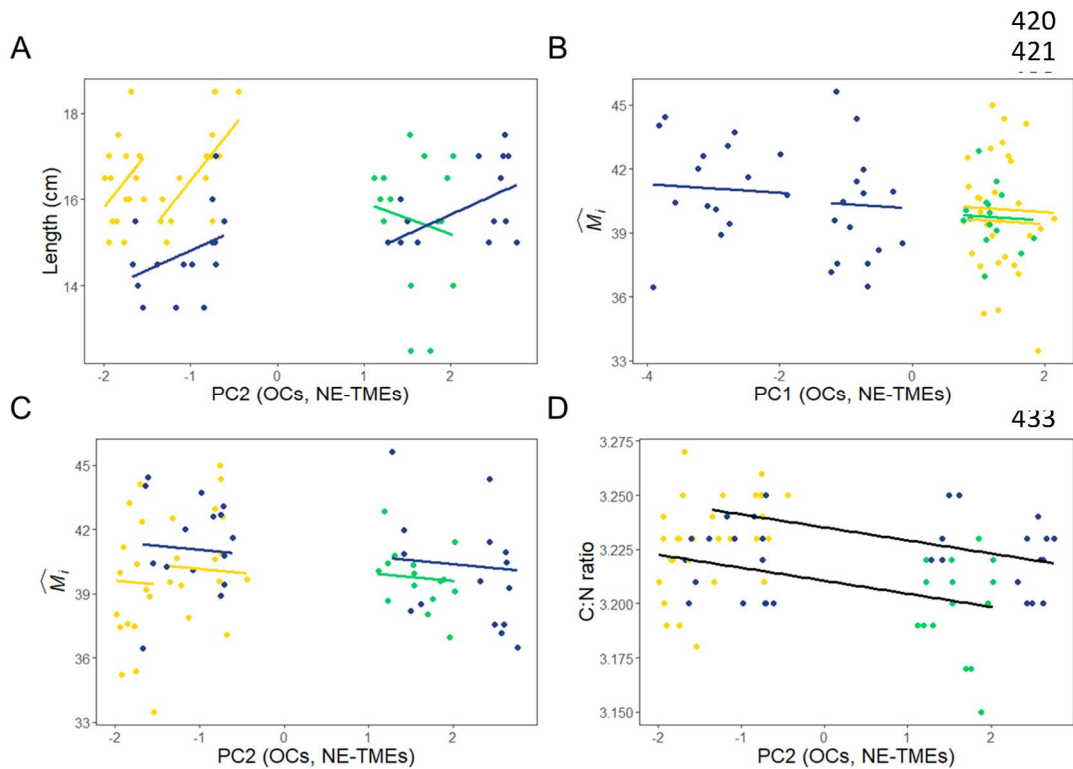
405 Figure 3: Effects of the principal components of trace metal elements (TMEs) on sea bass length
 406 (panel A), and body condition (\hat{M}_i , panels B and C). Gironde, Loire, and Seine nurseries are
 407 represented in green, yellow, and blue dots, respectively (in absence of difference among nurseries,
 408 the predicted values' line is represented in black). The fitted lines result from the averaging of model
 409 parameters with $\Delta AICc < 2$ are presented in panel A and the predicted values of the single best model
 410 are presented in panels B and C.

411

412 Table 3: Relative performance of models testing the effect of principal components of organic
 413 contaminants and non-essential trace metal elements on the length, body condition index (\widehat{M}_i), and
 414 C:N ratio of European sea bass juveniles. All models with $\Delta AICc < 2$ are presented along with the first
 415 model with $\Delta AICc > 2$. Table entries defined in Table 1.
 416

Response variable	Explanatory variables	K	LogLik	AICc	$\Delta AICc$	w_i
Length	PC2*Nursery+Year	9	-105.56	231.89	0.00	0.36
	PC2+Nursery	6	-110.39	234.02	2.13	0.13
\widehat{M}_i	PC1	4	-171.03	350.63	0.00	0.20
	PC2+Nursery+Year	7	-167.58	350.84	0.21	0.18
	PC1+Year	5	-170.63	352.14	1.50	0.09
	Null	3	-173.10	352.53	1.90	0.08
	PC1+PC2	5	-170.85	352.56	1.93	0.08
	PC1+PC2+Nursery+Year	8	-167.58	353.35	2.72	0.05
C:N ratio	PC2+Year	5	191.25	-371.63	0.00	0.50
	PC1+PC2+Year	6	191.43	-369.62	2.01	0.18

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 Figure 4: Effect of the principal components of organic contaminants and non-

446 essential trace metal elements (OCs, NE-TMEs) on sea bass length (panel A), body condition (\widehat{M}_i ,
 447 panels B and C), and muscles' C:N ratio (panel D). Gironde, Loire, and Seine nurseries are
 448 represented in green, yellow, and blue dots, respectively (in absence of difference among nurseries,
 449 the predicted values' line is represented in black). The fitted lines result from the averaging of model
 450 parameters with $\Delta AICc < 2$ or the predicted values of the single best model (panels A and D).

451 DISCUSSION

452 Overall, we found clear between nursery and between year differences in contamination
453 profiles and concentrations of sea bass juveniles from the three largest estuaries hosting
454 nurseries of France's western coast. TMEs were consistently negatively related to both
455 growth and body condition while the effects of organic contaminants were slightly weaker
456 and ambiguous (fish with greater OCs contaminations had higher growth and lower body
457 condition).

458 1- Chemical contamination profiles

459 Among nurseries, differences in TMEs concentrations were broadly consistent with the
460 documented historical contamination of the estuaries. For instance, previous studies
461 focussing on molluscs and sediments have reported high values of Ag in Seine (Chiffolleau et
462 al., 2005), Pb in Loire (Claisse, 1989; Couture et al., 2010), and Cd in Gironde (Claisse,
463 1989; Lanceleur et al., 2011). Similar differences were also reported in higher trophic levels
464 such as flounder *Platichthys flesus* (Kerambrun et al., 2013) and sea bass sampled near
465 these estuaries (Schnitzler et al., 2011). For instance, analysing muscles of larger and/or
466 older sea bass juveniles (mean body length = 31 ± 4.6 cm) sampled near the mouth of these
467 estuaries, Schnitzler et al. (2011) found that Pb concentrations were 1.25 and 2.5-fold higher
468 in Loire than in Seine and Gironde, results broadly similar to ours (2.8 and 1.6-fold higher in
469 2019). In the liver of flounders, Kerambrun et al. (2013) found *ca.* 3.3-fold higher Pb
470 concentrations in Loire than in Seine. These consistent differences in nurseries'
471 contamination profiles result from past and/or present industries that have historically
472 contaminated estuaries with these elements and site-specific geochemical backgrounds.
473 Moreover, the large oil refinery in Loire is a source of hydrocarbons or petroleum products
474 primarily responsible of V contaminations in this estuary (Schlesinger et al., 2017). In
475 absence of well-documented sources of metal contamination, the other nursery and/or year
476 differences for As, Fe, Hg, Mn, Mo, Zn, and REEs are harder to interpret.

477 Within each nursery, comparing our TME results with those of previous studies in fish
478 from the same estuaries should be done very cautiously as these analysed i) specific tissues
479 (i.e. muscle, liver, gills or kidneys; Durrieu et al. 2005, Schnitzler et al. 2011, Kerambrun et
480 al. 2013) whereas we analysed whole bodies; and/or ii) sea bass from other size classes (i.e.
481 Schnitzler et al. 2011); and/or iii) other species (i.e. Kerambrun et al. 2013). Tissue
482 differences (i.e. organotropism) are indeed well-documented for TME bioaccumulation in fish
483 (e.g. Durrieu et al. 2005; Chauvelon et al. 2019), which also depends on TMEs and species
484 (i.e. trends between tissues may differ), precluding any direct comparison of concentrations
485 between studies that have analysed different tissues. Variations of bioaccumulation within or
486 between species (at similar TME exposure) are also well documented (e.g. Burger and
487 Gochfeld 2011; Merciai et al. 2014). Therefore, only sampling of same cohort at older ages
488 (within the nursery and one recruited in the stocks) can enable us to rigorously determine the
489 degree to which contamination profiles changes over time in sea bass.

490 For OCs, PCBs were by far predominant in all nurseries. Despite their ban more than
491 30 years ago, PCBs are still major POPs in French coastal areas and particularly in the
492 Seine estuary, whose catchment area includes major industrial and urban activities (Tappin
493 and Millward, 2015). High PCB concentrations were also found in crustaceans or fish in
494 Seine (Bodin et al., 2007; Schnitzler et al., 2011) compared to other French coastal areas
495 such as Western Brittany (6-8 times lower in dw) and Gironde (2 times less in lipid weight,
496 lw). The $\Sigma 27$ PCB median concentrations reported in the muscle of sea bass by Schnitzler et
497 al. (2011) were 4500, 4217 and 2422 ng g⁻¹ lw in the Seine, Loire and Gironde respectively
498 versus 6602, 1081 and 2774 ng g⁻¹ lw respectively for the ones determined in our study, i.e.
499 in a similar order of magnitude. All PCB congeners were highly correlated and predominant
500 congeners were the most bioaccumulative and persistent ones (CB-153, CB-138, and CB-
501 180), indicating that these profiles reflect past inputs with similar sources in all nurseries
502 (profiles are consistent with those reported in sea bass from estuaries on the Atlantic
503 coastline; Schnitzler et al., 2011).

504 The comparison of our data with environmentally-relevant available thresholds gave
505 the following results. Of all the contaminants that we measured, only PCBs had published
506 Environmental Assessment Criteria (EAC, concentrations below which unacceptable
507 biological effects are unlikely to occur; Lyons et al., 2017). In Seine, the EACs were
508 exceeded in 100% of the samples for CB-52, -101, -118 and -153 and were exceeded in 4
509 and 1 samples out of 6 for CB-180 and -28, respectively. CB-118 concentrations were above
510 its EAC in all samples from Loire and Gironde, while CB-101 and -180 were above EACs in
511 all samples from the Gironde. We found that PFOS concentrations were on average 1.7
512 times greater than the Environmental Quality Standard (EQS) defined for biota in the
513 European Water Framework Directive (i.e. 9.1 ng g^{-1} wet weight). There are no EAC for
514 TMEs, but Hg has an EQS defined for biota in the European Water Framework Directive
515 ($\text{EQS}_{\text{Hg}} = 0.020 \text{ mg kg}^{-1} \text{ ww}$; European Commission 2013). After conversion of our data on a
516 wet weight basis, 100% of our samples exceeded this EQS_{Hg} .

517 There were clear nursery-specific contamination profiles in OCs, a result consistent
518 with the among-nursery differences observed for some TMEs (i.e. Ag, Pb and Cd, see
519 above), and with other studies showing the specificity of OCs contaminations (Deshpande et
520 al., 2015; Gerig et al., 2016; Vorkamp et al., 2012). In particular, sea bass juveniles sampled
521 in Gironde had high levels of DDTs and dieldrin, indicating that they were more exposed to
522 pesticides than juveniles of the other nurseries. Dieldrin contributed substantially to the total
523 concentration of OCPs in Gironde but it is unclear whether dieldrin originated from the
524 degradation of aldrin (undetected in these samples), or from its direct use in agriculture
525 (banned in France since 1972) or in pest control (authorized until 1992). Dieldrin's estimated
526 half-life in temperate soils is less than 5 years (Ritter et al., 1998) and 10 years in fish
527 (Carlson et al., 2010) but the recent findings of residues of this banned pesticide in
528 vegetables from the Gironde region (Gironde prefecture, 2016) suggests that there are
529 probably some contemporary inputs. Despite its phase-out in 2002 and its inclusion in the
530 Stockholm convention (2009), PFOS concentrations were comparable to those measured in
531 other estuarine fish species in Europe (Zafeiraki et al., 2019), suggesting high persistence of

532 this OC and/or contemporary use of PFOS precursor compounds (Benskin et al., 2013). The
533 less studied long chain PFCAs were also ubiquitous despite their recent addition to the
534 candidate list of 'Substances of Very High Concern' under the European REACH regulation.

535 In addition to these differences among estuaries, we found substantial variation
536 among years. In Seine, all targeted OCs levels in 2018 were two-fold higher than in 2019.
537 Such large inter-annual variations in CC have previously been observed (McLeod et al.,
538 2014; Williams and McCrary, 2021) and can be explained by variations in CC inputs, or in
539 other environmental factors (such as temperature) that can affect fish ability to eliminate CC
540 (McLeod et al., 2014). For instance, the lower river flows in Seine and Loire in 2019
541 compared to 2018 could have induced lower inputs via flooding and/or sediment
542 remobilisation, hence leading to lower contamination levels in fish.

543

544 2- Relationships between chemical contaminants and growth and body condition

545 We found no evidence of deficiencies in the essential TMEs we measured, either because
546 their concentrations were too high to lead to deficiencies or because the projection of E-
547 TMEs and NE-TMEs on the same PCs may prevent us from detecting any deficiency in E-
548 TMEs. Conversely, we found that juvenile sea bass with high PC1 values (i.e. high
549 concentrations of Ag, As, Co, Cu, Mo, and Zn) were smaller. Increasing values of PC1 were
550 also associated with lower body condition suggesting that these TMEs could be involved in
551 major physiological constraints in this species. We found no relationship between PC2 (i.e.
552 high levels of Fe, Pb, V, and REE) and growth, but a clear negative relationship with fish
553 body condition. As body condition is more sensitive to environmental variations than body
554 growth, this result suggests that these TMEs might have exerted weaker physiological
555 constraints on sea bass juveniles either because of more efficient regulation/detoxification
556 mechanisms (Wang and Rainbow, 2010) or because of lower concentrations of these TMEs
557 in the environment. Finally, PCs were not related to the C:N ratio, a measure reflecting the
558 amount of lipids in animal tissues (rich in C) relative to proteins (rich in N). The C:N ratio

559 values were low and their variance across our samples was very small (range: 3.15-3.50) for
560 a fish species (Hoffman et al., 2015) and probably reflect the low quantity of lipids (including
561 fatty acids) in muscles of sea bass juveniles. A low variation in the response variable can
562 dampen our ability to detect relationships with moderate effect sizes and might explain the
563 lack of congruence between the C:N ratio and the morphometric condition index (\widehat{M}_i).
564 Broadly, these results are congruent with both experimental and field studies that showed for
565 instance that the presence or high concentrations of NE-TME such as Cd can lead to a
566 decline in growth, in energy reserves (lipid storage) and hence body condition (e.g. Pierron et
567 al. 2007), especially if combined to other environmental stressors such as temperature
568 variations (Petitjean et al., 2020).

569 In the PCA including OCs and NE-TMEs, fish with higher growth had higher values of
570 PC2 (i.e. high concentrations in dieldrin, Σ DDT, Σ PCB, and Σ vlc-PFCA). These OCs are not
571 expected to have any positive effect on growth but this result could reflect a higher resource
572 requirement of fish with high growth. These requirements entail an increase in the quantity of
573 ingested OCs, which may not yet have any detrimental effect on growth. As the OCs
574 associated with PC2 are primarily lipophilic, they may be harder to excrete and hence more
575 concentrated in large fish. Similarly to TMEs, we found that measures of body condition were
576 consistently more related to the OCs as both PC1 and PC2 were negatively related to \widehat{M}_i and
577 PC2 was negatively related to the C:N ratio (Fig.4). This again could suggest that the
578 presence of OCs induces energetic costs that might lead to a decline in body condition. Two
579 fish species from a polluted bay in Brazil also had lower body condition in comparison with
580 reference sites, an observation related to the levels PFASs (Hauser-Davis et al., 2021). If
581 direct effects of PFASs on fish growth generally occurred in laboratory-based experiments at
582 concentrations above environmental levels (Ankley et al., 2021), PFASs could have a
583 stronger effect on body condition by disrupting metabolic pathways (Lee et al. 2020). The
584 strong relationship between the C:N ratio and PC2 can also reflect the lipophilicity of the OCs
585 that define this principal component axis.

586

587 3- Perspectives and conclusions

588 Measuring the effect of chemical contaminants on fish life history traits in the wild is
589 challenging for two main reasons: measurements of contaminants can only be carried out in
590 individuals that survived to the time of sampling, and individuals are exposed to a wide
591 variety of contaminants whose interactions can influence their relationships with growth
592 and/or body condition. Indeed, determining whether chemical contaminants and the survival
593 of individuals are related in the field or documenting accurately the time of death of
594 individuals is challenging. Even when possible (e.g. in tagged individuals), any spatio-
595 temporal lag between the exposure of individuals to contaminants and their time of death
596 might weaken any links between the two processes. Failing to account for differences in the
597 survival rates of individuals may lead to underestimations of effects of chemical contaminants
598 on juvenile fish populations (i.e. missing fraction issue; Grafen 1988). Moreover, bringing
599 together CCs that have very different concentrations and measuring their effect on fish life
600 history traits is challenging because there are many other confounding factors and potentially
601 other contaminants that can have be related to juveniles' growth and body condition, making
602 it harder to pinpoint an overall effect. Finally, it is particularly difficult to fully combine TMEs
603 and OCs as quantification of OCs requires a greater amount of tissues and a substantially
604 more demanding analytical effort. This forced us to pool fish and lose some statistical power
605 by decreasing individuals' variation in OC concentrations and growth/body condition.

606 In spite of these limitations, we found that some TMEs and OCs were clearly
607 negatively related to the growth and body condition of juvenile sea bass. The presence of
608 persistent OCs that have been banned sometimes for several decades is worrying as it
609 suggests a long-lasting deterioration of the estuaries and chronic exposures of juvenile fish.
610 As both bioaccumulation and biodilution may occur, it is now critical to quantify changes in
611 concentration and harmfulness of these contaminants in older juveniles and in reproductive
612 adults, to better understand their long-term consequences for individuals in terms of survival
613 and reproductive success and hence understand their population-level consequences.

614

615 **Ethical approval**

616 Authorization and ethical approval for fish sampling provided by national (DPMA) and
617 regional authorities (Normandie, Pays de la Loire, Nouvelle Aquitaine); National & regional
618 committees of professional fishermen (CNPMM, CRPM Normandie; COREPMM Pays de
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622

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629

630 **CRedit Authorship**

631 Christophe Lebigre: Conceptualization, Data curation, Formal analysis, Funding acquisition,
632 Investigation, Methodology, Software, Validation, Visualization, Writing - original draft,
633 Writing - review & editing.

634

635 Tiphaine Chouvelon, Yann Aminot & Catherine Munsch: Conceptualization, Data curation,
636 Formal analysis, Funding acquisition, Investigation, Methodology, Project administration,
637 Resources, Validation, Writing - review & editing.

638

639 Mickaël Drogou, Ronan Le Goff & Nicolas Briant: Data curation, Investigation, Resources,
640 Validation, Writing - review & editing.

641

642

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