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

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

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

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

MATERIAL AND METHODS

1- Studied nurseries and sample collection

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

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

2- Sample preparation

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

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

3- Growth and body condition indices

As all sampled fish were 1-year-old, differences in their length reflected differences in growth. There were slight initial differences in juvenile length among sites, fish being slightly longer in Loire compared with Seine and Gironde estuaries (mean ± SD; Gironde: 15.50 ± 1.57; Loire 16.51 ± 0.94; Seine 15.41 ± 0.97 cm; ANOVA: $F_{2,102}$ = 13.30, P < 0.001). To estimate fish body condition, we used a morphometric parameter (the Scaled-Mass Index $\widehat{M}_i$; Peig and Green, 2009) and a biochemical parameter (the C:N ratio). $\widehat{M}_i$ is a morphometric condition index parameterised using individuals' length (L_i) and body weight (M_i) and more weakly affected by differences in body size than other morphometric indices (Peig and Green, 2010). $\widehat{M}_i$ 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 relationship estimated by a standardized major axis (SMA) regression between the logarithms of body weight and size, and L_0 a reference size (i.e. the arithmetic mean length calculated across all sampled individuals). The C:N ratio is also a proxy of body condition, reflecting the lipid content of tissues (Hoffman et al., 2015; Post et al., 2007). The portion of muscle samples collected for C and N analyses was weighed (0.40 ± 0.05 mg dry weight) and C and N contents were measured using a Thermo Scientific Flash EA1112 elemental analyser.

4- Trace metal elements analyses

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

ALTEC AMA-254). Measurements were carried out by strictly following the standard operating procedure described in US-EPA method 7473 (U.S. Environmental Protection Agency, 1998). We then measured concentrations of E-TMEs (arsenic (As), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), vanadium (V), and zinc (Zn)) and NE-TMEs (silver (Ag), cadmium (Cd), mercury (Hg), lead (Pb), and 14 elements of the Rare Earth Elements (REE) family (lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu)). Aliquots (*ca.* 200 mg of homogenised powder) were placed in Teflon bombs and mineralized with ultra-pure HNO_3 acid and water using a microwave system (ETHOS-UP, Milestone). Finally, digests were diluted to 50 mL with ultra-pure water and the concentration of TMEs were quantified by inductively coupled plasma mass spectrometry (ICP-MS, ICAP-Qc ThermoFisher). The quality assurance of all TME analyses relied on blanks, internal standard controls and on the accuracy and reproducibility of data relative to certified reference materials (CRM). Blank values were systematically below detection limits and CRM values concurred with certified concentrations. The CRM used were IAEA-407 (fish homogenate, International Atomic Energy Agency/IAEA) and IAEA-142 (mussel homogenate, IAEA) for Hg; IAEA-407, DORM-4 (fish protein, National Research Council of Canada/NRCC) and DOLT-5 (dogfish liver, NRCC) for other TMEs; BCR-668 for REE (mussel tissue, Joint Research Centre of the European Commission). Detection limits and average recovery rates are detailed in Supp. Table 1. All TMEs are reported in µg g^{-1} of dry weight (dw), except REE which were reported in ng g^{-1} dw.

5- Organic contaminant analyses

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

2). The analyses of PCBs and OCPs were performed using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS, Hewlett-Packard 6890 gas chromatograph coupled to a Micromass AutoSpec Ultima mass spectrometer), and PFASs were measured using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MSMS, Acquity UPLC coupled to Xevo® TQ-S micro, Waters Corp.). For each of the 15 pools of samples (75 individuals), we measured 18 different PCBs including the 6 indicator PCBs (i-PCBs) and the 12 dioxin-like PCBs (dl-PCBs; detailed in Supp. Table 3). All PCBs were summed and referred to as ∑PCB hereafter. Among OCPs, we measured dieldrin and 5 different DDT isomers (detailed in Supp. Table 3); these isomers were also summed and are referred to ∑DDT hereafter. Among PFASs, we focussed on the C_4- to C_{10}-perfluoroalkyl sulfonates (PFSAs) and C_6- to C_{14} perfluorocarboxylic acids (PFCAs). As the perfluorooctane sulfonate (PFOS) accounted for over 90% of the total concentrations of PFSAs in all samples, we decided to focus on this compound among PFSAs. Moreover, to keep the number of variables low, we decided to focus on perfluorononanoic acid (PFNA; C_9 PFCA), and the sum of C_{12}-C_{14} PFCAs, referred to as the very long chain PFCAs (∑vlc-PFCA) as PFNA and Σvlc-PFCA showed peculiar profiles in our samples. We carried out all OC analyses following strict QA/QC procedures, i.e. clean and low-dust atmosphere, positive pressure laboratories, in-house quality control samples, procedural blanks, quantifications using external calibration, addition of labelled compounds before extraction to calculate recoveries, and participation in the Quasimeme interlaboratory comparison tests for the marine environment with satisfactory Z-scores (i.e. between -1.2 and +0.2 for OCPs, -1.2 and 0.0 for PCBs and -0.9 and 0.0 for PFASs). Detailed information on QA/QC performances can be found in Supp. Table 3 and Supp. Table 4. All OCs were measured in ng g^{-1} dw.

6- Statistical analyses

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

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

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

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

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

RESULTS

1- Concentrations and profiles of chemical contaminant in sea bass

Concentrations varied according to nurseries and/or years for all TMEs except Cr (Supp. Table 5). There were significant differences among nurseries and years in As, Fe, Hg, Mn, Pb, V, and ∑REE (Supp. Table 5, concentrations reported in the text are median values). More specifically, concentrations in As were higher in Seine than in Loire in 2018 (4.20 *vs* 2.54 mg kg^{-1} dw) but its overall concentration increased in 2019 (5.59 mg kg^{-1} dw) with no significant differences among nurseries (Supp. Table 5). Conversely, concentrations in Fe in Loire 2019 increased compared with the previous year (from 53.6 to 61.0 mg kg^{-1} dw) and compared to other sites whose concentrations did not change (Supp. Table 5). Concentrations of Hg were lower in 2018 in Loire (0.156 mg kg^{-1} dw) but increased subsequently to reach similar concentrations to those of fish sampled in Gironde and Seine in 2019 (0.216 mg kg^{-1} dw, Supp. Table 5). The concentrations of Mn were lower in Seine 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 ∑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 (∑PCB > 100 ng g^{-1}
312 dw), followed by PFOS and ∑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 ∑PCB levels was found among nurseries and between
317 years (Supp. Table 6). ∑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 ± 3%) and *p,p'*-DDD (15 ± 2%). We measured high
321 concentrations of dieldrin in sea bass juveniles sampled in Gironde (dieldrin/∑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 ∑vlc-PFCA to the overall concentration in PFCAs was 51

± 2%, 40 ± 4%, and 69 ± 5% in Gironde, Loire and Seine, respectively. The ratios of ∑PFCA/PFOS clearly differed in Loire where PFOS levels were relatively higher, indicating different sources of PFASs in this estuary.

2- Multivariate analyses

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

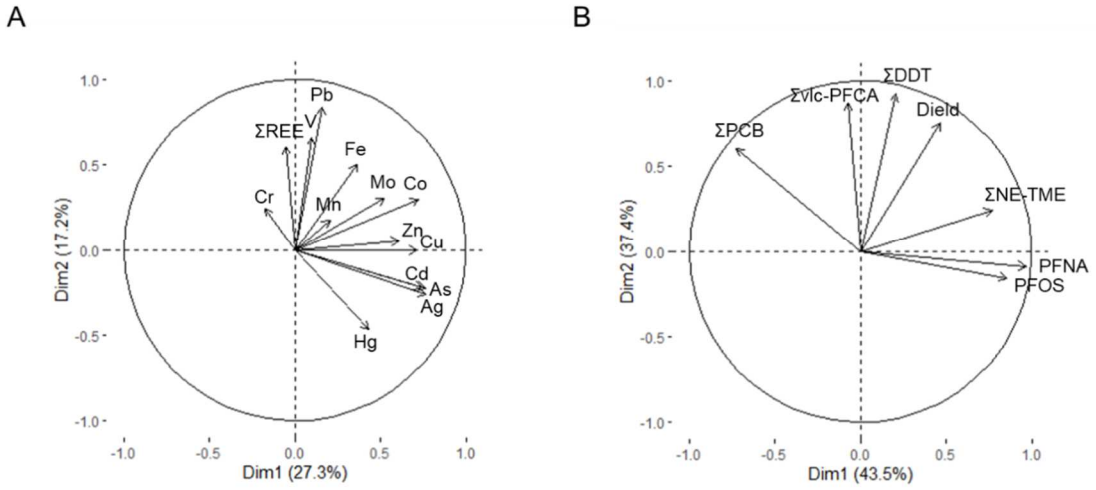

Figure 1: Projection of the different trace metal elements (TMEs; panel A) and organic contaminants with non-essential metal trace elements (OCs, NE-TMEs; panel B) on the first two axes of the separate principal component analyses. See Supp. Table 5 for contaminants' abbreviations.

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).

357

358

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

Dependent variable	Model	K	LogLik	AICc	ΔAICc	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

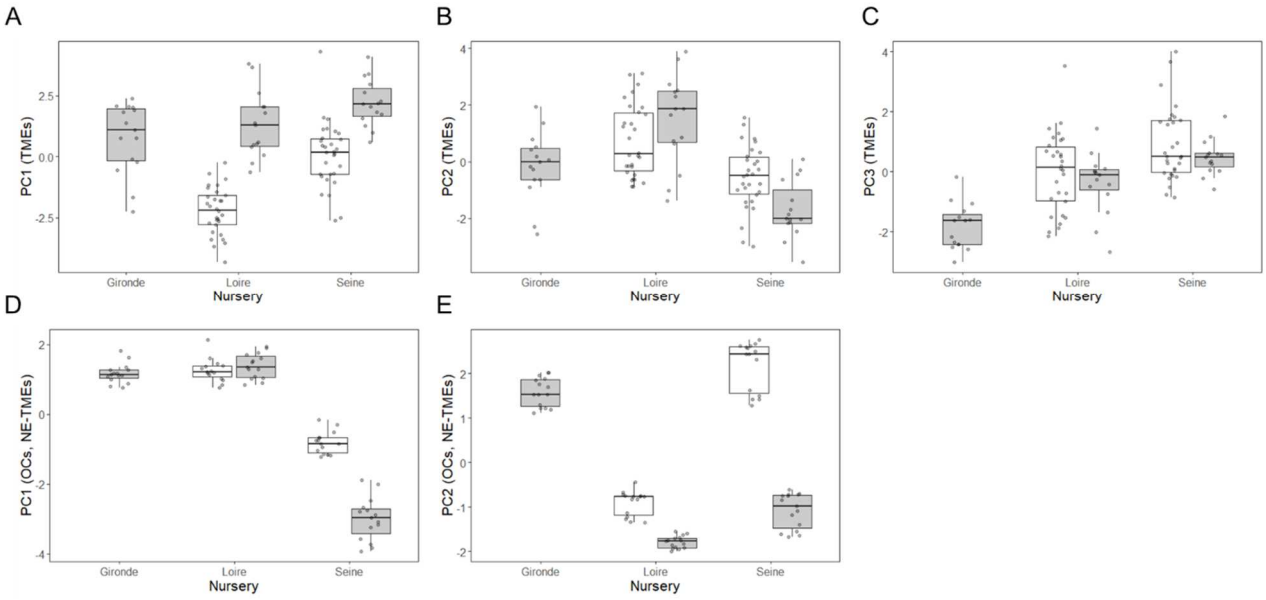

Figure 2: Differences among nursery and year in the principal components extracted for trace metal elements (TMEs) alone (panels A-C) and organic contaminants with non-essential trace metal elements (OCs, NE-TMEs, panels D and E). White and grey boxes represent samples collected in 2018 and 2019, respectively.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

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 ∑DDT, dieldrin, ∑PCB, and ∑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 ΔAICc < 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

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

Response variable	Explanatory variables	K	LogLik	AICc	ΔAICc	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
$\widehat{M}_i$ 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

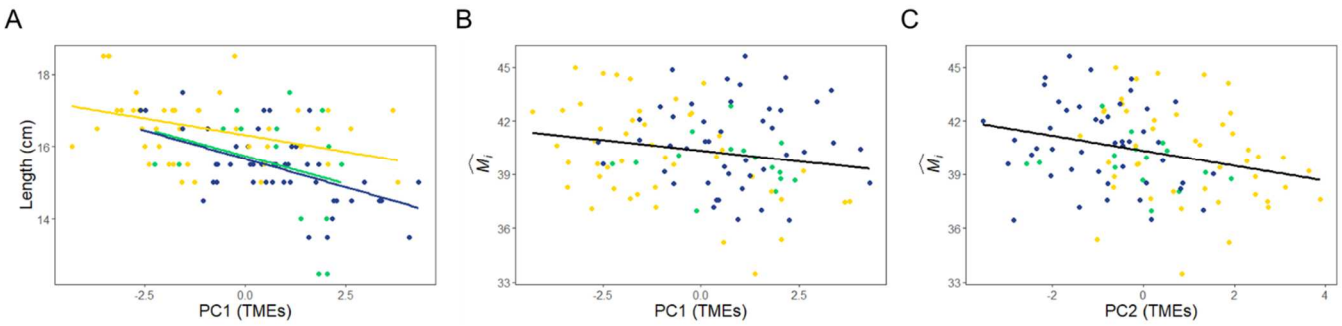

Figure 3: Effects of the principal components of trace metal elements (TMEs) on sea bass length (panel A), and body condition ($\widehat{M}_i$, panels B and C). Gironde, Loire, and Seine nurseries are represented in green, yellow, and blue dots, respectively (in absence of difference among nurseries,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

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

Response variable	Explanatory variables	K	LogLik	AICc	ΔAICc	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

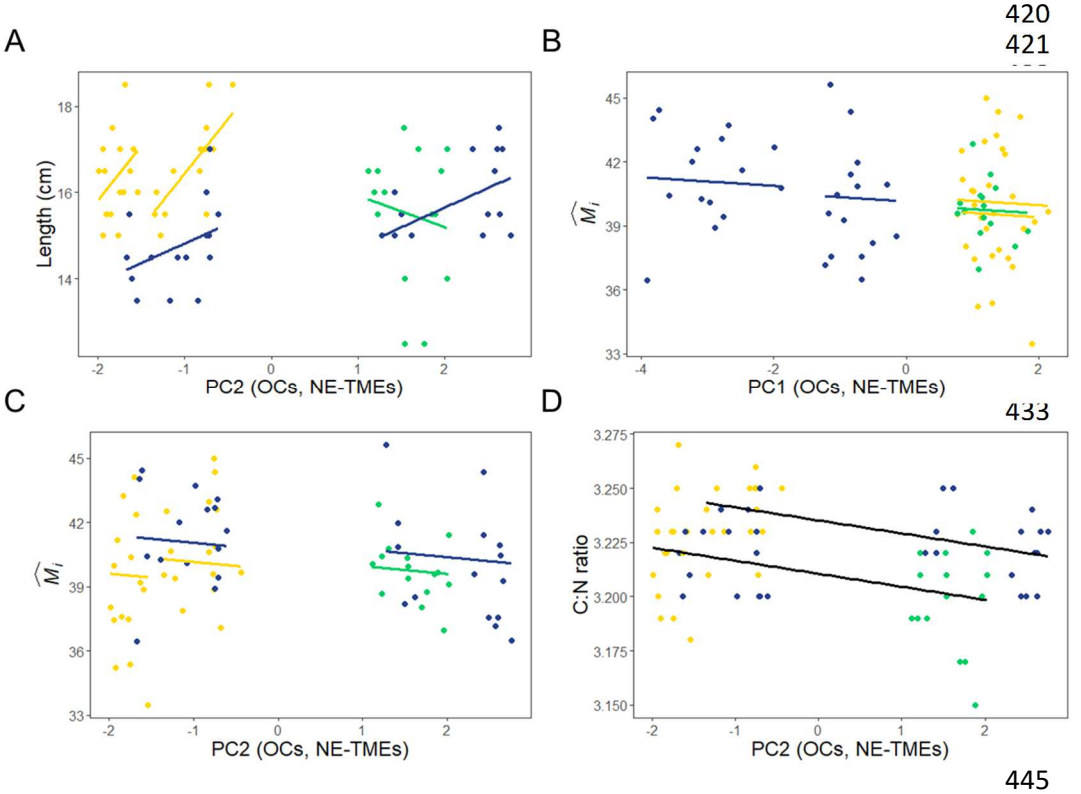

Figure 4: Effect of the principal components of organic contaminants and non-essential trace metal elements (OCs, NE-TMEs) on sea bass length (panel A), body condition ($\widehat{M}_i$, panels B and C), and muscles' C:N ratio (panel D). Gironde, Loire, and Seine nurseries are represented in green, yellow, and blue dots, respectively (in absence of difference among nurseries, the predicted values' line is represented in black). The fitted lines result from the averaging of model parameters with ΔAICc < 2 or the predicted values of the single best model (panels A and D).

DISCUSSION

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

1- Chemical contamination profiles

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

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

For OCs, PCBs were by far predominant in all nurseries. Despite their ban more than 30 years ago, PCBs are still major POPs in French coastal areas and particularly in the Seine estuary, whose catchment area includes major industrial and urban activities (Tappin and Millward, 2015). High PCB concentrations were also found in crustaceans or fish in Seine (Bodin et al., 2007; Schnitzler et al., 2011) compared to other French coastal areas such as Western Brittany (6-8 times lower in dw) and Gironde (2 times less in lipid weight, lw). The Σ27 PCB median concentrations reported in the muscle of sea bass by Schnitzler et al. (2011) were 4500, 4217 and 2422 ng g^{-1} lw in the Seine, Loire and Gironde respectively versus 6602, 1081 and 2774 ng g^{-1} lw respectively for the ones determined in our study, i.e. in a similar order of magnitude. All PCB congeners were highly correlated and predominant congeners were the most bioaccumulative and persistent ones (CB-153, CB-138, and CB-180), indicating that these profiles reflect past inputs with similar sources in all nurseries (profiles are consistent with those reported in sea bass from estuaries on the Atlantic 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 (EQS_{Hg} = 0.020 mg kg^{-1} 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

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

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

2- Relationships between chemical contaminants and growth and body condition

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

3- Perspectives and conclusions

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

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

Ethical approval

Authorization and ethical approval for fish sampling provided by national (DPMA) and regional authorities (Normandie, Pays de la Loire, Nouvelle Aquitaine); National & regional committees of professional fishermen (CNPMEM, CRPM Normandie; COREPMEM Pays de la Loire, CRPMEM Nouvelle Aquitaine) for 2018 (Ref. 18/2 216 097 AVT1) and 2019 (Ref. Osiris PFEA400018DM0310001; ref. Ifremer: 18/2216441). All fish analysed were dead by the time of tissue sampling.

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CRediT Authorship

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

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

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

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