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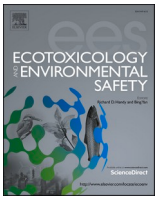

Effects of metals and persistent organic pollutants on the fitness and health of juveniles of the endangered european sturgeon *Acipenser sturio* Exposed to W1ater and sediments of the garonne and dordogne rivers

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ABSTRACT

The last remaining population of European sturgeon (*Acipenser sturio*) lives in the Gironde-Garonne-Dordogne (France) catchment (GGD). Captive young individuals are released into the GGD hydrosystem each year, as part of a restocking programme. This study aims to assess the health status of juveniles *A. sturio* to current conditions in the GGD hydrosystem, to evaluate their capacity to survive and grow in a moderately anthropized ecosystems. 3-month-old farmed sturgeons were exposed for one month in experimental conditions that mimic the environmental conditions in the Garonne and Dordogne rivers, followed by five months of depuration. After one month of exposure, fish exposed to Dordogne and Garonne waters bioaccumulated higher levels of metals and persistent organic pollutants, displayed a reduced hepato-somatic index, and had depleted levels of lipids and glycogen content in their liver, when compared with the Reference group. However, metabolic and swimming performance, as well as the costs of swimming were not impaired. After the 5 months depuration, a significant decrease of K was observed for all exposure conditions. HSI also decreased with time. The overall health status and adaptive capacity of juvenile *A. sturio* appeared to be maintained over the experimental 6 months' period. Juveniles of *A. sturio* seem to have the adaptive capacity to survive and grow in the GGD hydrosystem, after being released as part of a restocking programme.

1. Introduction

The European sturgeon (*Acipenser sturio*) is the most threatened migratory diadromous fish in Europe (IUCN, 2016). Classed as a critically endangered species by the International Union for the Conservation of Nature (IUCN), populations of *A. sturio* decreased dramatically during the 20th century principally due to human activities (Lepage et al., 2000). The last wild population of only a few thousand individuals exists in the Gironde Garonne Dordogne (GGD) basin in France (Williot and Castelnaud, 2011a; Williot et al., 2011b). The last natural reproduction of the species was observed in 1994 (Rochard et al., 2001). This species is strictly protected by many conventions and European directives (Rochard et al., 2011). A restoration program adopted by the Bern Convention was launched with managed stocking operations in the GGD basin, to ensure conservation (Rosenthal et al., 2007). In 1994, a captive brood stock was established from wild adults at the INRAE experimentation station in St Seurin sur l'Isle (GGD catchment, France), to secure the species' future and provide a basis for subsequent releases into the wild (Williot et al., 2007). Since 2007, more than 1.6 million juveniles have been produced and released to sustain the natural

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population (Acolas et al., 2012). Males become mature between 8 and 12 years and females between 13 and 16 years old (Rochard et al., 2011). 3 months old individuals becoming mature are released and expected to re-enter the GGD basin for reproduction in the next decade. To protect and manage this species, it is essential to have more precise information on the risks and the environmental conditions faced by these released juveniles sturgeons in the GGD basin. While the GGD catchments have been considered as an ecological reference for many years, historical contamination by polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), organochlorine pesticides (OCPs) and various metals has nevertheless been reported (Maury-Brachet et al., 2008). Pollutants are known to impact fish physiology (Videler, 1993; Claireaux and Lefrançois, 2007; Javed and Usmani, 2019). PAHs (Vignet et al., 2014a; Marit and Weber, 2012; Claireaux and Lefrançois, 2007), organochlorines (Bussolaro et al., 2012) and metals (Javed and Usmani, 2019; Turan et al., 2020) are known to induce oxidative stress, genotoxicity and histopathology changes which can result in developmental defects, reduce aerobic metabolism and growth, as well as impairing reproduction.

In this context, improving knowledge on the sensitivity of the European sturgeon to the current water quality conditions found in the GGD basin appears particularly relevant for the protection and restoration of this endangered species. This will contribute to analysis of European sturgeons' ability to t progressively recolonize the GGD catchment.

The consequences of environmental constraints on overall health status can be evaluated through assessment of physiological performance, supported mainly by aerobic metabolic scope (AMS; Fry, 1947). AMS is related to the instantaneous aerobic metabolic rate an organism may allocate to its energy-demanding activities (e.g. locomotion, digestion, feeding, reproduction). It represents the organism's capacity to supply oxygen for these activities. AMS is therefore claimed by different authors to be a relevant proxy of fitness, and thus a good indicator of an organism's capacity to survive and reproduce in a given environment (e.g. Fry, 1947, Claireaux and Lefrançois, 2007). Studies about aerobic metabolisms are often associated with swimming performance, assessing the physiological consequences of certain environmental constraints (Domenici et al., 2013). Locomotion is indeed an important trait that underlies essential functions such as foraging, escape from predators, and migration (Hammer, 1995; Videler, 1993). Critical swimming speed U_{crit} (i.e. the maximal velocity a fish can reach during a swimming step protocol; Brett, 1964, Hammer, 1995) is frequently considered an indicator of swimming capacities in fish coping with a given environment (e.g.Videler, 1993, Hammer, 1995). In addition, while U_{crit} partly relies on the anaerobic metabolism, it is also closely related to AMS, since it depends on the maximum ability of fish to provide oxygen during a sustained swimming activity. Furthermore, metabolic and swimming performance are both known to be modulated by a set of environmental parameters, such as temperature, dissolved oxygen (Claireaux and Lagardère, 1999) and pollutants (Marit and Weber, 2012; Lucas et al., 2016).

The present study was designed to characterize the metabolic and physiological responses of *A. sturio* juveniles in order to evaluate their capacity to adapt to the physical and chemical conditions of the Garonne and Dordogne Rivers. The GGD catchment was chosen because (i) it is the natural habitat of the last wild European sturgeon population (Williot and Castelnaud, 2011a; Williot et al., 2011b), (ii) the sturgeon restocking program has been carried out in this area since 1995 supported by release of captive juveniles and (iii) the next natural reproduction of *A. sturio* is expected in this area (Acolas et al., 2012; Delage, 2015). Based on previous environmental analysis, which evaluated the quality of different spawning grounds (Delage, 2015), two sites were selected for more intensive investigations: Pessac-sur-Dordogne in the Dordogne River, and La Réole in the Garonne River. Environmental conditions of both release sites were reproduced in experimental dynamic mesocosms. After a one-month exposure, the physiological status of juvenile European sturgeons was estimated through the assessment of condition index, aerobic metabolic, histopathology (liver) and swimming performance, as well as the related cost of transport.

2. Materials and methods

2.1. Fish and condition index

Sturgeon juveniles were obtained from assisted reproduction of the French captive stock at the INRAE experimentation station in St Seurin sur l'Isle (France; Williot et al., 2011b). Larvae were reared at 18 °C, fed daily with *Artemia nauplii* for 3 weeks following hatching, and then with chironom larvae until the experiments were carried out. When aged 1.5 months, fish were individually tagged under electronarcosis using PIT-tags (FDX Biolog id 10,224,8 mm × 1.4 mm, 0.031 g). Tags were inserted into the intraperitoneal thanks to an incision with the tip of a needle. Incisions were sutured with surgical glue.

Until the age of 2.5 months, fish were stocked in a 1.100 L tank within a recirculating bio-filtered system, with water between 17.5 and 19 °C, in the dark. After this period, the photoperiod was kept at a cycle of 12 h/12 h day and night for the duration of the experiments. When the fish were 3 months old (T_0), pools of 100 fish were formed and then exposed for a duration of 1 month (T_1) to 3 exposure conditions: a control, as well as two conditions that mimic 2 release areas (i.e. Dordogne and Garonne). They were then depurated for 5 months (T_6). Fish were euthanized and dissected to sample and weigh their livers. From these biometrics, Fulton's condition factor (i.e. factor K) was assessed at T_0, T_1 and T_6 by the formula of Htun-Han (1978):

$$K = 100 \cdot W/L^3 \quad (1)$$

Where W is the weight of the fish (in g) and L the total length (in cm).

Hepato-somatic index was also calculated at T_0, T_1 and T_6 using the formula:

$$HSI = (W_{liver}/W_{gutted}) \times 100 \quad (2)$$

Where W_{liver} is the weight of the liver (in g) and W_{gutted} is the fish gutted weight (in g).

2.2. Exposure conditions

The three batches of one hundred 3 month-old fish were then transferred into three identical annular flumes called SCOLA (Structure expérimentale d'étude du COmportement LArvaire, Fig. S1) for one month. SCOLA mimicked the environmental conditions (hydrodynamics, temperature, oxygen, photoperiod, contamination) of the two release sites: Pessac-sur-Dordogne in Dordogne (France; GPS XRGF93 45°95′72.16′′; YRGF93 63°91′359.09′′) and La Réole in Garonne (France; GPS XRGF93 46°84′76.34′′; YRGF93 64°17′855.65′′, France). This experiment also included a control treatment with reference water (i.e. water used at the experimentation station of St Seurin sur l'Isle for rearing fish) and reference sediment (Fontainebleau sand). Before the experiment, 1 m^3 sediment from the Dordogne and from the Garonne rivers was collected downstream from the release area, transported in a wash boiler and added into the SCOLAs. Water was renewed in each SCOLA twice a week. For that purpose, 1 m^3 of water was sampled at each of the release sites twice a week, and transported in a tank to the laboratory. Reference water was directly pumped in to the station from a natural resource. This renewal was expected to overcome the potential degradation of the most labile organic pollutants, and thereby to standardize the conditions to which the fish were exposed to while in the SCOLA. In the 3 SCOLA, fish were exposed to a continuous current of 0.27 ± 0.03 $m.s^{-1}$. Water quality was assessed daily and was characterized by 18 ± 0.5 °C, oxygen concentration of 9.15 ± 0.3 ppm, pH of 7.55 ± 0.07, salinity of 0 and a photoperiod of 12 h/12 h day and night. Ammonium and nitrate were remained within recommended ranges

([NH_4^+]<0.1 mg.l^{-1}, [$NO3^-$]$_R$=9.88 ± 8.0 mg.l^{-1}, [$NO3^-$]$_G$=19.35 ± 5.51 mg.l^{-1}, [$NO3^-$]$_D$=16.47 ± 3.77 mg.l^{-1}, [$NO2^-$]<0.5 mg.l^{-1}. During all the experiment, fish were fed daily with chironom larvae at 30% of their weight.

2.3. Chemical analysis

2.3.1. Sediment analysis

Chemical analyses were performed at T_0 and T_1 month on the sediments collected in situ, i.e. at the moment of their sampling on the two selected release sites and 1 month after their transfer to SCOLA. Samples were freeze-dried and sieved at 2 mm. Chemical analyses focused on 17 polycyclic aromatic hydrocarbons (PAHs), 8 congeners of polychlorinated biphenyls (PCBs), 13 organochlorinated pesticides (OCPs) and 11 metals (Table 1, Table S1). All the components and their concentrations are detailed in the supplementary material (Table S1, S2). Most of the substances are recognized as priority compounds by the International Council for Exploration of the Sea (ICES), the US Environmental Protection Agency (EPA) and the E.U. Directive for water protection and management (2000/60/EC).

2.3.1.1. Organic pollutants. PAHs, PCBs and OCPs analyses were performed as described previously (Budzinski et al., 2000; Barhoumi et al., 2014). Sediment samples were extracted using microwave assisted extraction (Budzinski et al., 2000) and extract clean-up was performed on a multilayer column (alumina and silica with activated copper). Extracts were then split into two fractions: fraction 1 was analyzed for PAHs (see below) while fraction 2 was purified on acid-impregnated silica prior to organochlorine analysis. PAHs were analyzed by gas chromatography-mass spectrometry in Selected Ion Monitoring mode (GC-MS; HP 5890 Series II, MSD 5972, Agilent Technologies, Palo Alto, CA, USA), through isotope dilution quantification (Baumard and Budzinski, 1997). OCPs and PCBs were quantified by gas chromatography coupled with electron capture detection (GC-ECD) (Barhoumi et al., 2014) using internal standard quantification with PCBs not present in environment (CBs 30, 103, 155, 198) and 4,4′-DDT-d8 as internal standards. Procedural blanks were performed for each sample batch and blank-correction was applied. Samples and blanks were spiked with internal recovery standards prior to extraction procedures and with a syringe standard (octachloro-naphthalene) before injection to monitor Internal standard (IS) losses; recoveries higher than 60% were accepted. Limits of detection (LODs) and limits of quantification (LOQs) were set as the concentrations yielding signal-to-noise ratios of 3 and 10, respectively. LOQs were in the range 0.1–0.2 ng g^{-1} dw for OCPs, 0.1–0.2 ng g^{-1} dw for PCBs and 0.5–2 ng g^{-1} dw for PAHs.

2.3.1.2. Metals. Sediment digestions for trace metal analyses were performed on representative subsamples (~30 mg of dry, powdered and homogenized sediment) in closed 50 mL-polypropylen reactors (DigiTUBEs®, SCP SCIENCE) in a heating block (2 h at 110 °C) with 1.5 mL HCl (12 M Suprapur®, Merck), 0.75 mL HNO3 (14 M) and 2.5 mL HF (22 M), as previously detailed. After evaporation to dryness (10 h at 110 °C), re-dissolution of the residues was performed with 0.25 mL HNO3 (14 M) and 5 mL Milli-Q® water. After cooling, the solution was brought to 10 mL using Milli-Q water. Trace metal concentrations were measured by ICP-MS (X7 Series 2, Thermo®) performed with external calibration under standard conditions. The analytical method was quality checked with international certified reference materials (NIST 8704, BCR 277R, NCS DC 73308) in order to verify accuracy and precision. Theanalytical method was checked for quality by parallel analysis of international certified reference materials (SLRS-4; SLRS-5, LGC, TM-RAIN 4). Accuracies were ≥ 92% for all trace metal analysed with precision ≤ 5% (RSD). Fifteen metals (Ag, As, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sn, Sb, Th, V, U and Zn) were analysed.

2.3.2. Water samples analysis

2.3.2.1. Quantification of pesticides. Time-integrated dissolved pesticide concentrations were estimated using polar organic chemical integrative samplers (POCIS), used for two weeks in our SCOLAs (october 2014) and in the Dordogne and Garonne rivers (Belles et al., 2014). The extraction procedure for POCIS was previously described (Belles et al., 2014). Briefly, the POCIS sorbent (i.e. Oasis HLB) was transferred into an empty glass Solid Phase Extraction (SPE) tube with polyethylene frits and dried under vacuum for 30 min. Analytes were then eluted successively with 10 mL of MeOH, 10 mL of MeOH/DCM (50/50, v/v) and 10 mL of DCM in a receiving vial that contained internal standards. The extract was concentrated to 300 µL (MeOH) under nitrogen flow and stored at − 20 °C until further analysis.

Following that, the cartridge was eluted in the same way as described earlier. A blank procedure was also performed in order to assess possible contamination.

Analyses were carried out using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analytical system consisted of an

Table 1
Sum of mean concentrations of organic and metallic pollutants in sediment (ng.g^{-1} dw) and water (ng.l^{-1}) at T_0, i.e. when the 3 month-old fish were transferred in the SCOLA and at T_1, i.e. at the end of the one month- experimental duration.

	Time of exposure	Treatment	∑ PAHs	∑ PCBs	∑ OCPs	∑ pesticides (POCIS)	∑ metals
Sediment in SCOLA (ng.g^{-1} dw)	T_0	Reference	3.67	0.26	0.22	–	0.74 10^5
		Dordogne	147.32	0.39	0.29	–	4.52 10^5
		Garonne	28.07	0.33	0.41	–	1.15 10^5
	T_1	Reference	2.66	0.27	0.21	–	–
		Dordogne	76.37	0.00	0.14	–	5.33 10^5
		Garonne	7.81	0.69	0.40	–	4.35 10^5
Water in SCOLA (ng.l^{-1})	T_0	Reference	–	–	–	14.1	9.34 10^3
		Dordogne	–	–	–	84.8	8.98 10^3
		Garonne	–	–	–	541.9	6.20 10^3

No stastistical analyses were applied on the data because for some organic chemicals we have only one measure per condition and point of sampling. Means±SD. ∑ PAHs: Naphtalene, Acenaphthylene, Acenaphtene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)Anthracene, Chrysene + Triphenylene, Benzo(b+j + k)Fluoranthene, Benzo(e)pyrene, Benzo(a)pyrene, Perylene, Indeno(c-d)Pyrene, Dibenzo(a,h + a,c)Anthracene, Benzo(ghi)Perylene. ∑ PCBs: CB 50, CB 28, CB 52, B 101, CB 118, CB 153, CB 138, CB 180. ∑ OCPs: HCB, Gamma HCH, Heptachlore, Heptachlore hepoxide, 2,4′ DDE, cis chlordane, trans nonachlor, 4,4′ DDE, 2,4′ DDD, 4,4′ DDD, 2,4′ DDT, 4,4′ DDT, Mirex. ∑ Pesticids: Average concentrations was calculated over the exposure period i.e. one month, the compounds were: 124 DCPU,134 DCPU,1343 DCPMU, acetochlore, acetochlore ESA, acetochlore OA,alachlore, amethryne, atrazine, atrazine 2 hydroxy, azoxystrobine; carbendazime; carbetamide; carbofuran; chlorotoluron; chlorsulfuron; cyanazine; cyromazine; DEA; DIA; dichlofluanide; diflufénican; dimetachlore; dimethoate; diuron; DMSA; DMST, fenarimol; flazasulfuron; fluazifop-p-butyl; flusilazole; foramsulfuron; fosthiazate; hexazinone; hydroxy simazine; imidaclopride; irgarol; isoproturon; linuron; metalaxyl m; metamitrone: metazacchlore; methiocarbe; metolachlore; metolachlore ESA; metolachlore OA; metoxuron; monolinuron; nicosulfuron; norflurazon; prochloraz; promethryne; propachlore; propazine; prosulfuron; pymethrozine; quizalofop-ethyl; quizalofop-p-tefuryl; simazine; terbutryne; terbutylazine; terbutylazine desethyl; thiamethoxan; tolylfluanide. ∑ metals: V, Cr, Co, Ni, Cu, Zn, As, Ag, Cd, Al, Pb

Agilent LC 1290 infinity LC system coupled to a 6460 triple quadrupole and equipped with an electrospray ionization source (ESI) used in positive mode (adapted from Belles et al., 2014). Data acquisition was performed in multiple reaction mode (MRM). Extraction recoveries were assessed using HLB sorbent spiked with analytes (100 ng each) and varied from 80% to 126%. LOQs were in the range 0.05 – 0.25 ng L^{-1}. For each series of analysis, procedural blanks were analysed (complete procedure but without matrix). Control calibrating standards were also injected every 15 samples, and injection of analytical blanks was also performed in combination with the analysis of procedural blanks. Chemical analyses focused on 57 pesticids. All the components and their concentrations are detailed in the Supplementary material (Table S3).

2.3.2.2. Quantification of metals. Water samples from SCOLA and from the Dordogne and Garonne rivers were filtered using 0.2 μm Sartorius polycarbonate filters. Filtrates were collected in 30 cc polypropylene bottles, previously decontaminated and thoroughly rinsed with the filtrate (HNO_3 suprapur grade; 1:1000) and stored at 4 °C until analysis. Metal analysis was done by ICP-MS (Thermo, Thermo X II series) under standard conditions. Methodological blanks, using ultrapure water, were systematically below detection limits. Our analytical method was checked for quality by parallel analysis of international certified reference materials (SLRS-4; SLRS-5). Accuracy was within 8% of the certified values and reproducibility was better than 5%. All the components and their concentrations are detailed in the Supplementary material (Table S4).

2.3.3. Fish tissues analyses

Quantification of pollutants was performed on the target tissues in fish in order to evaluate bioaccumulation at T_0, T_1 and T_6. To perform tissue analyses, some *A. sturio* juveniles were euthanized by decapitation, and dissection was performed in order to sample the three target organs: whole livers, dorsal muscles and total gill arches from both sides. All the components and their concentrations are described in Table 2 and in supplementary material Table S5.

2.3.3.1. PCBs and OCPs quantification. Analyses were performed on freeze-dried tissues according to the analytical protocol described in Bodin et al. (2014). Samples were frozen at − 40 °C and then water was eliminated under vacuum (0,1 mbar) using LL3000 (Heto PowerDry LL3000, 3Kg/24 H) freeze dryer system. Freeze-drying was stopped when the temperature reached 0 °C. Dry tissues were initially ground. Freeze-dried liver tissues were ground with agate mortar,while muscle tissue was ground using "Tube Mill control" milling system from IKA®. Dry tissues were ground first. The 8 PCBs and 13 OCPs analyzed in the sediment were also analyzed in the liver and muscles of 4 fish randomly sampled at T_0 and T_1, as described in Section 2.3.1.

PAHs concentration was not measured, since they are quickly and efficiently metabolized by the P450 system in fish, particularly in sturgeon and, consequently, are hardly detectable in tissue, and not at all representative of exposure. Biota samples were analyzed using the same protocol described above for sediment analysis (Supplementary material and methods 1) but using accelerated solvent extraction instead of microwave-assisted extraction. Fish organs were analyzed individually and LOQs were in the range 0.1–1.0 ng g^{-1} dw for OCPs and 0.1–5 ng g^{-1} dw for PCBs.

2.3.3.2. Metals quantification. Eleven metals were measured (Ag, Al, As, Cd, Co, Cr, Cu, Ni, Pb, V and Zn) in 3 fish tissues: gills, liver and muscles (Table 1, Table 3, Table S5). Samples were analysed by inductively coupled optical emission spectrometry (ICP-OES 720, Agilent Technologies). Depending on the samples and quantity of tissue available, fish were analysed individually or in pools. For T_0, 8 fish were analysed individually. For T_1 and T_6, 4 pools of 5 individuals each (n = 20) were analysed for each condition. Samples were frozen at −20 °C until analysis. Before digestion, tissue samples were dried at 45 °C for 48 h and weighed. The tissues were first digested during 3 h at 100 °C (hot block CAL 3300, Environmental Express, USA) after addition of nitric acid 63% (3 mL for 100 mg of dry tissue, Merck, Darmstadt, Germany) in polypropylene tubes. Samples were then diluted 6-fold with pure water (Milli-Q, Bedford, MA, USA). The validity of the method was periodically checked with Tort-2 and Dolt-4 certified material (lobster hepatopancreas and dogfish liver from NRCC-CNRC, Ottawa, Canada) and values obtained were within the certified ranges (data not shown). Blank digest was also carried out in the same way.

2.4. Histopathological evaluation of liver

Liver samples were collected from 30 juveniles at T_1 and from other 30 juveniles at T_6. Samples were fixed in 4% formalin and then dehydrated in graded ethanol solutions and embedded in paraffin. 4 μm-thick sections were routinely stained with haematoxylin-eosin-saffron (HES) and additional sections were stained with Periodic Acid Schiff (PAS) for glycogen. Liver sections were observed microscopically and a semi-quantification using a 5-grade scale which was performed to document the intensity of lipid and glycogen storage into hepatocytes. A pathologist certified by the European College of Veterinary Pathology reported histological lesions. Hepatocytic storage was scored semi-quantitatively using a 5-grade (0 = no lesion, 1 =minimal, 2 =mild, 3 =marked and 4 =severe lesion).

2.5. Aerobic metabolic scope and critical swimming speed

2.5.1. Experimental set-up

Two identical swimming tunnels (LoligoApS., Denmark) were employed to simultaneously challenge two fish. The swimming tunnel allowed fish locomotor activity to be controlled, and for associated oxygen consumption to be measured. Each swimming tunnel was composed of a 10 L respirometer containing a swimming chamber (40 x l0×10 cm) where fish were placed for testing. A motor fitted with a three bladed propeller generated the water flow. Some deflectors and a plastic honeycomb promoted laminar flow with a uniform velocity profile (vertical and horizontal). Each swimming respirometer was submerged in the buffer tank where the reference water temperature (i.e. 18 °C) and oxygen (i.e. 100% air saturation) was controlled using a thermoregulator and air pump. The fish's oxygen consumption (MO_2 in mg $O_2.g^{-1}.h^{-1}$) was measured by intermittent-flow respirometry (Lucas et al., 2014a). Water supply in each swimming respirometer was provided by flush pumps, which controlled water flow from the buffer tank to the swimming respirometer. This allowed alternation between (a) phases of water and oxygen renewal and (b) phases where the swimming respirometer was isolated and MO_2 measured, with a cycle of 5: 20 min respectively. The oxygen probe (i.e. optic fiber; Presens, Germany) was introduced in the swimming chamber and connected to an oxymeter (Microx, Presens, Germany) to record the level of dissolved oxygen in the water every 5 s using the software Oxyview (Presens, Germany). The probe was calibrated once at the beginning of the swim test using 0% and 100% air saturation with a controlled temperature at 18 °C; no derivation was observed during the duration of the experiment.

2.5.2. Experimental protocol

After the 30 days of exposure (T_1) in SCOLA, *A. sturio* juveniles were successively transferred into a 1 m^3 circular tank where they were starved for 24 h before being challenged (n = 11 for Dordogne, n = 12 for Garonne, n = 7 for the Reference). Each experimental trial consisted of simultaneously challenging two fish that had been exposed to one of the three experimental conditions: Reference, Dordogne and Garonne. Each individual was gently transferred from the tank to the swimming respirometer and its total length was measured. Fish were then left undisturbed for a recovery night, with a water flow of 0.5 $BL.s^{-1}$ (i.e. Body Length per second). During the swimming challenge, water flow

Table 2
Concentration of PCBs (ng.g^{-1} dw) and OCPs (ng.g^{-1} dw) in liver and muscle of juveniles of *Acipenser sturio* at T_0, i.e. when the 3 month-old fish were transferred in the SCOLA and at T_1, i.e. at the end of the one month-experimental duration.

Tissue	Time of exposure	Treatment	CB 50	CB 28	CB 52	CB 101	CB 118	CB 153	CB 138	CB 180	∑PCBs
Liver (ng.g^{-1} dw, n = 4)	T_0	Reference	1.41 ± 0.39	3.07 ± 0.42	15.37 ± 2.48	21.82 ± 5.58	22.27 ± 4.15	16.53 ± 2.88	11.58 ± 1.93	11.13 ± 1.74	**103.18 ± 13.90**
	T_1	Reference	1.83 ± 0.21	2.57 ± 0.58	15.50 ± 0.91	23.44 ± 1.59	19.60 ± 1.20	17.94 ± 0.64	12.40 ± 0.16	11.91 ± 0.95	**105.16 ± 1.34**
		Dordogne	1.99 ± 0.86	2.34 ± 0.88	15.77 ± 4.73	29.65 ± 5.95	30.80 ± 8.55	33.28 ± 5.32	25.50 ± 4.99	16.71 ± 1.40	**154.88 ± 23.67**
		Garonne	1.71 ± 0.58	1.84 ± 0.86	13.34 ± 3.12	23.40 ± 2.83	21.75 ± 1.82	21.50 ± 1.77	15.30 ± 1.42	12.74 ± 1.19	**111.58 ± 7.75**
Muscles (ng. g^{-1} dw, n = 4)	T_0	Reference	0.40 ± 0.04	0.70 ± 0.10	3.55 ± 0.63	5.23 ± 0.53	5.10 ± 0.5	4.71 ± 0.37	3.41 ± 0.27	2.84 ± 0.43	**25.95 ± 2.65**
	T_1	Reference	0.28 ± 0.04	0.50 ± 0.06	2.44 ± 0.40	3.29 ± 0.88	3.56 ± 0.75	3.01 ± 0.51	2.31 ± 0.37	2.13 ± 0.60	**17.51 ± 2.94**
		Dordogne	0.34 ± 0.11	0.45 ± 0.12	2.17 ± 0.65	3.73 ± 0.87	4.48 ± 1.46	4.59 ± 0.68	3.59 ± 0.61	2.43 ± 0.35	**21.79 ± 4.76**
		Garonne	0.27 ± 0.08	0.42 ± 0.10	2.06 ± 0.66	3.03 ± 0.78	3.39 ± 0.57	3.49 ± 0.52	2.75 ± 0.40	2.62 ± 0.35	**18.03 ± 3.09**

Tissue	Time of exposure	Treatment	HCB	Gamma HCH	Heptachlor	Heptachlor epoxide	2,4′-DDE	cis-chlordane	trans-nonachlor	4,4′-DDE	2,4′-DDD	4,4′-DDD	2,4′-DDT	4,4′-DDT	Mirex	∑OCPs
Liver (ng.g^{-1} dw, n = 4)	T_0	Reference	8.91 ± 3.63	1.86 ± 0.33	<LOQ	0.92 ± 0.58	1.50 ± 0.71	< LOQ	2.69 ± 0.58	57.44 ± 13.51	0.64 ± 0.14	5.43 ± 0.88	0.95 ± 0.16	0.30 ± 0.09	3.67 ± 0.60	**84.08 ± 17.58**
	T_1	Reference	7.63 ± 2.48	2.06 ± 0.16	<LOQ	1.19 ± 0.16	1.23 ± 0.24	< LOQ	3.11 ± 0.78	50.98 ± 6.48	0.72 ± 0.06	6.10 ± 0.29	1.12 ± 0.09	0.26 ± 0.08	4.61 ± 0.31	**78.90 ± 10.33**
		Dordogne	4.97 ± 2.72	2.07 ± 0.52	<LOQ	0.75 ± 0.62	0.27 ± 0.53	< LOQ	6.83 ± 3.83	53.13 ± 19.57	3.07 ± 1.05	9.37 ± 0.66	2.29 ± 0.73	0.74 ± 0.18	5.33 ± 0.97	**88.63 ± 29.46**
		Garonne	4.48 ± 2.00	1.98 ± 0.48	<LOQ	0.95 ± 0.37	0.62 ± 0.21	< LOQ	6.31 ± 2.26	49.58 ± 14.25	1.16 ± 0.28	7.27 ± 1.07	1.28 ± 0.06	0.50 ± 0.15	4.86 ± 0.57	**79.00 ± 21.22**
Muscles (ng. g^{-1} dw, n = 4)	T_0	Reference	1.56 ± 0.20	0.75 ± 0.17	<LOQ	0.22 ± 0.03	< LOQ	< LOQ	0.61 ± 0.20	14.43 ± 2.59	< LOQ	1.28 ± 0.13	0.23 ± 0.01	< LOQ	0.77 ± 0.11	**19.89 ± 3.35**
	T_1	Reference	1.99 ± 0.10	0.53 ± 0.06	<LOQ	0.24 ± 0.02	< LOQ	< LOQ	0.62 ± 0.08	11.23 ± 0.22	< LOQ	0.96 ± 0.05	< LOQ	< LOQ	0.86 ± 0.08	**16.43 ± 0.32**
		Dordogne	2.07 ± 0.20	0.43 ± 0.10	<LOQ	0.18 ± 0.05	< LOQ	< LOQ	1.23 ± 0.58	9.75 ± 1.87	0.43 ± 0.12	1.00 ± 0.29	< LOQ	< LOQ	0.99 ± 0.14	**16.14 ± 3.30**
		Garonne	2.56 ± 0.63	0.53 ± 0.15	<LOQ	0.23 ± 0.05	< LOQ	< LOQ	1.53 ± 0.47	14.22 ± 3.13	0.22 ± 0.03	1.22 ± 0.29	< LOQ	< LOQ	1.07 ± 0.12	**21.52 ± 4.50**

Means±SD. LOQ = limit of quantification, LD = limit of detection. Concentrations were assessed on 4 replicates for each condition of exposure. No statistical analyses were applied on the data because for some organic chemicals we have only one measure per condition and point of sampling.

Table 3
Concentration of metals in liver and muscle of juveniles of Acipenser sturio at T_0, i.e. when the 3 month-old fish were transferred in the SCOLA and at T_1, i.e. at the end of the one month- experimental duration and T_6, i.e. after 5 months-depuration.

Tissue	Time of exposure	Treatment	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	V	Zn	∑metals
Liver (ng. g^{-1} dw)	T_0	Reference	870 ± 230 (n = 6)^{a}	< LD	510 ± 40 (n = 6)^{ab}	< LD	170 ± 30 (n = 6)^{a}	380 ± 40 (n = 6)^{a}	14,100 ± 1900 (n = 6)^{a}	269.9 ± 21.6 (n = 6)^{a}	2280 ± 160 (n = 6)^{a}	< LD	160 ± 100 (n = 4)^{ab}	2420 ± 230 (n = 6)^{a}	30 ± 10 (n = 2)^{a}	54,480 ± 4760 (n = 6)^{a}	**345,300 ± 29,100**
	T_1	Reference	1390 ± 160 (n = 4)^{ab}	2840 ± 1200 (n = 4)^{a}	470 ± 340 (n = 4)^{ab}	<LD	360 ± 10 (n = 4)^{ab}	360 ± 70 (n = 4)^{a}	21,960 ± 2440 (n = 4)^{b}	381,900 ± 22250 (n = 4)^{ab}	2040 ± 190 (n = 4)^{a}	80 ± 10 (n = 4)^{ab}	180 ± 100 (n = 2)^{ab}	3310 ± 190 (n = 4)^{a}	260 ± 10 (n = 4)^{ab}	73,960 ± 7670 (n = 4)^{ab}	**489,110 ± 34,640**
		Dordogne	1650 ± 190 (n = 4)^{ab}	2580 ± 950 (n = 4)^{a}	660 ± 50 (n = 3)^{ab}	< LD	510 ± 60 (n = 4)^{bc}	510 ± 160 (n = 4)^{a}	21,760 ± 1680 (n = 4)^{b}	570,440 ± 49,560 (n = 4)^{bc}	2560 ± 340 (n = 4)^{a}	140 ± 30 (n = 4)^{a}	370	3320 ± 320 (n = 4)^{a}	430 ± 100 (n = 4)^{cb}	87,400 ± 6900 (n = 4)^{b}	**691820 ± 60340**
		Garonne	1550 ± 1700 (n = 4)^{ab}	1680 ± 570 (n = 4)^{a}	760 ± 160 (n = 4)^{a}	< LD	530 ± 110 (n = 4)^{bc}	320 ± 50 (n = 4)^{a}	23,660 ± 2130 (n = 4)^{b}	578,290 ± 10,0480 (n = 4)^{bc}	2650 ± 450 (n = 4)^{a}	140 ± 30 (n = 4)^{a}	5	3760 ± 780 (n = 4)^{a}	450 ± 80 (n = 4)^{cb}	92,650 ± 10,950 (n = 4)^{b}	**706445 ± 115,960**
	T_6	Reference	1.75 ± 0.38 (n = 10)^{ab}	2.54 ± 0.19 (n = 10)^{a}	0.43 ± 0.07 (n = 8)^{a}	0.01 ± 0.003 (n = 2)^{a}	0.73 ± 0.09 (n = 4)^{a}	0.14 ± 0.02 (n = 9)^{b}	24.5 ± 4.05 (n = 10)^{a}	962.03 ± 133.46 (n = 10)^{a}	2.16 ± 0.25 (n = 10)^{a}	0.12 ± 0.05 (n = 5)^{ab}	0.17 ± 0.04 (n = 8)^{a}	4.86 ± 0.58 (n = 10)^{a}	0.82 ± 0.09 (n = 10)^{a}	91.16 ± 9.34 (n = 10)^{a}	**1091.42 ± 148**
		Dordogne	1.62 ± 0.21 (n = 10)^{ab}	2.18 ± 0.32 (n = 10)^{a}	0.47 ± 0.07 (n = 10)^{a}	0.01 ± 0.002 (n = 7)^{a}	0.67 ± 0.1 (n = 10)^{a}	0.2 ± 0.03 (n = 10)^{b}	23.4 ± 2.4 (n = 10)^{a}	836.18 ± 104.33 (n = 10)^{ab}	2.00 ± 0.15 (n = 10)^{a}	0.17 ± 0.03 (n = 8)^{a}	0.13 ± 0.04 (n = 6)^{a}	5.43 ± 0.66 (n = 10)^{a}	0.64 ± 0.05 (n = 10)^{ab}	87.03 ± 8.31 (n = 10)^{a}	**960.13 ± 117**
		Garonne	1.88 ± 0.19 (n = 10)^{a}	2.43 ± 0.38 (n = 10)^{a}	0.63 ± 0.12 (n = 10)^{a}	0.03 ± 0.02 (n = 5)^{a}	0.72 ± 0.06 (n = 10)^{a}	0.39	25.17 ± 2.76 (n = 10)^{a}	748.68 ± 78.90 (n = 10)^{ab}	2.50 ± 0.23 (n = 10)^{a}	0.06 ± 0.02 (n = 5)^{b}	0.23 ± 0.06 (n = 9)^{a}	4.41 ± 0.36 (n = 10)^{a}	0.81 ± 0.09 (n = 10)^{a}	98.01 ± 7.01 (n = 10)^{a}	**885.95 ± 90**
Muscles (ng.g^{-1} dw)	T_0	Reference	20	702 ± 320 (n = 5)^{a}	510 ± 30 (n = 6)^{ab}	1 ± 0 (n = 2)^{a}	13 ± 4 (n = 4)^{a}	870 ± 120 (n = 6)^{a}	1730 ± 80 (n = 6)^{abc}	23,750 ± 1670 (n = 6)^{ab}	1050 ± 50 (n = 6)^{a}	50 ± 20 (n = 5)^{bc}	60 ± 10 (n = 4)^{a}	3130 ± 120 (n = 6)^{ab}	73 ± 13 (n = 5)^{a}	27,770 ± 1170 (n = 6)^{a}	**59,747 ± 3610**
	T_1	Reference	20 ± 15 (n = 2)	580 ± 17 (n = 3)^{ab}	570 ± 50 (n = 4)^{bc}	3 ± 0 (n = 2)^{ab}	23 ± 10 (n = 3)^{a}	720 ± 70 (n = 4)^{a}	2060 ± 80 (n = 4)^{abc}	21,400 ± 1300 (n = 4)^{abc}	960 ± 30 (n = 4)^{a}	60 ± 5 (n = 3)^{abc}	70 ± 10 (n = 2)^{a}	2850 ± 110 (n = 4)^{b}	37 ± 6 (n = 4)^{a}	25,990 ± 1210 (n = 4)^{a}	**55,343 ± 3070**
		Dordogne	70 ± 10 (n = 3)	1110 ± 260 (n = 4)^{b}	640 ± 30 (n = 4)^{c}	7 ± 2 (n = 3)^{ab}	9 ± 2 (n = 3)^{a}	810 ± 60 (n = 4)^{a}	2160 ± 190 (n = 4)^{ab}	27290 ± 550 (n = 4)^{a}	1010 ± 40 (n = 4)^{a}	120 ± 20 (n = 4)^{a}	< LD	3410 ± 70 (n = 4)^{a}	30 ± 10 (n = 4)^{ab}	27,870 ± 1640 (n = 4)^{a}	**64,536 ± 2882**
		Garonne	50 ± 29 (n = 2)	860 ± 140 (n = 4)^{ab}	53 ± 30 (n = 4)^{abc}	9 ± 2 (n = 2)^{ab}	14 ± 7 (n = 3)^{a}	940 ± 130 (n = 4)^{a}	2450 ± 300 (n = 4)^{a}	24,110 ± 1600 (n = 4)^{ab}	890 ± 70 (n = 4)^{a}	110 ± 40 (n = 4)^{ab}	< LD	3110 ± 290 (n = 4)^{a}	26 ± 11 (n = 4)^{ab}	24,750 ± 1200 (n = 4)^{ab}	**57,849 ± 3580**
	T_6	Reference	0.017 ± 0.016 (n = 2)^{a}	0.64 ± 0.16 (n = 10)^{a}	0.39 ± 0.04 (n = 10)^{c}	0.015 ± 0.004 (n = 7)^{a}	0.02 ± 0.005 (n = 5)^{a}	0.40 ± 0.03 (n = 10)^{b}	1.59 ± 0.16 (n = 10)^{cd}	14.85 ± 1.96 (n = 10)^{c}	0.61 ± 0.03 (n = 10)^{b}	0.05 ± 0.004 (n = 5)^{ab}	0.14 ± 0.04 (n = 2)^{a}	3.24 ± 0.13 (n = 10)^{b}	0.07 ± 0.03 (n = 2)^{ab}	17.56 ± 1.47 (n = 10)^{b}	**39.592 ± 4**
		Dordogne	0.03	0.91 ± 0.31 (n = 10)^{a}	0.63 ± 0.04 (n = 10)^{a}	0.007 ± 0.001 (n = 7)^{abc}	0.023 ± 0.007 (n = 6)^{a}	0.43 ± 0.06 (n = 10)^{b}	1.76 ± 0.20 (n = 10)^{bc}	19.63 ± 3.38 (n = 10)^{bc}	0.68 ± 0.05 (n = 10)^{b}	0.03 ± 0.01 (n = 7)^{b}	0.06 ± 0.02 (n = 6)^{b}	3.97 ± 0.09 (n = 10)^{a}	0.04 ± 0.01 (n = 9)^{ab}	17.1 ± 2.00 (n = 10)^{b}	**45.3 ± 6**
		Garonne	< LD	0.56 ± 0.11 (n = 10)^{a}	0.66 ± 0.07 (n = 10)^{a}	0.012 ± 0.003 (n = 7)^{ab}	< LD	0.46 ± 0.12 (n = 10)^{b}	1.22 ± 0.18 (n = 10)^{d}	17.86 ± 2.03 (n = 10)^{bc}	0.70 ± 0.03 (n = 10)^{b}	0.03 ± 0.004 (n = 5)^{b}	0.13 ± 0.01 (n = 6)^{a}	3.39 ± 0.08 (n = 10)^{b}	0.02 ± 0.01 (n = 5)^{b}	17.17 ± 0.9 (n = 10)^{b}	**42.212 ± 4**

Means±SD. LOQ = limit of quantification, LD = limit of detection. The numbers of replicates used for each component per condition of exposure are indicated in bracket. Different letters indicate significant differences between times and conditions for each organ (Kruskal-Wallis, $p < 0.05$).

was increased by step of 0.5 BL.s^{-1}. Each step lasted 20 min, during which fish MO_2 was measured. The experiment stopped when the fish fatigued, i.e. when it could no longer swim against the current and fell against the grid at the rear of the swimming chamber and the speed was quickly decreased to 0.5 BL.s^{-1}. After a recovery period, fish were then removed from the swimming respirometer. Standard length ($SL_{Reference}$=15.2 ± 1.3, $SL_{Dordogne}$=15.8 ± 0.8, $SL_{Garonne}$=15.1 ± 0.9), total length ($TL_{Reference}$=18.1 ± 1.5, $TL_{Dordogne}$=19.4 ± 1.1, $TL_{Garonne}$=18.5 ± 0.9) and mass of each individual ($M_{Reference}$=23.0 ± 6.3, $M_{Dordogne}$=24.1 ± 4.6, $M_{Garonne}$=21.3 ± 3.5) were measured. Before and after each trial, a blank measurement was carried out to quantify microbial oxygen consumption in the swimming respirometer. The average of these two values was subtracted from the measured oxygen consumption, each fish was tested once. Standard metabolism will be estimated in resting individuals (i.e. when swimming activity is null), while active metabolism will be assessed in individuals swimming at their critical swimming speed.

2.5.3. Calculations

2.5.3.1. Critical swimming speed. The critical swimming speed U_{crit} was calculated according to the formula of Brett (1964):

$$U_{crit}=U_t+t_1 \cdot t^{-1} \cdot U_1 \quad (3)$$

Where U_t (BL.s^{-1}) is the highest velocity maintained for an entire step, t_1 (min) is the time spent until exhaustion is reached in the last step, t (min) is the swimming period for each step and U_1 is the increment velocity (i.e. in the present study 20 min and 0.5 BL.s^{-1}, respectively).

2.5.3.2. Oxygen consumption (MO_2). Oxygen consumption MO_2 is expressed in mg O_2.g^{-1}.h^{-1} and calculated using the following formula:

$$MO_{2meas}=\Delta[O_2] \cdot \Delta t^{-1} \cdot V \cdot M_{meas}{}^{-1} \quad (4)$$

where $\Delta[O_2]$ (mg O_2. L^{-1}) is the variation in oxygen concentration during the measurement period Δt (in h), V (in L) is the volume of the respirometer minus the volume of the fish and M_{meas} (in g) is the fish measured mass.

2.5.3.3. Standard metabolic rate, active metabolic rate and aerobic metabolic scope. The model used to describe the relationship between MO_2 and U was the hydrodynamics-based power function (; Videler, 1993; Claireaux et al., 2006). The regression equation was fitted using the following equation and least squares methods:

$$MO_2=a+bU^{c-1} \quad (6)$$

where MO_2 is the oxygen consumption (mg O_2.g.$^{-1}$.h^{-1}), a is the SMR, b and c are two constants and U is the swimming speed (in BL.s^{-1}).

This equation allowed assessment of AMR as the maximum oxygen consumption measured during the swimming challenge and SMR with regard to the constant a in the equation, i.e. MO_2 when swimming speed was null. AMS is the difference between AMR and SMR. SMR, AMR and AMS were assessed for each individual fish.

2.5.3.4. Cost of transport. The cost of transport (COT, J.kg^{-1}.m^{-1}) is a proxy of the swimming efficiency of fish. The lower the COT is the more efficient is the locomotion. MO_2 values were converted to J.kg^{-1}.m^{-1} using an oxycalorific equivalent (3.24 cal.mg O_2 $^{-1}$; Beamish, 1978) and converting cal to J (4.18 J.cal^{-1}). The obtained values were divided by the corresponding swimming speed (m.s^{-1}). The relationship between COT and U was plotted and a regression was fitted using the following equation (Videler, 1993):

$$COT=MO_2 \cdot U^{-1} \text{ or } a \cdot U^{-1}+bU^{(c-2)} \quad (7)$$

It leads to a curve with a minimum at the optimal swimming speed (U_{opt} in m.s^{-1}). U_{opt} was calculated according to the following equation (Videler, 1993; Claireaux et al., 2006):

$$U_{opt}=[a/(c\text{-}1)b]^{1/c} \quad (8)$$

In a similar way, the net cost of transport (COT_{net}, J.kg^{-1}.m^{-1}) was calculated according to the following equation (Videler, 1993; Claireaux et al., 2006):

$$COT_{net}=MO_{2net} \cdot U^{-1} \quad (9)$$

Where MO_{2net} in J.kg^{-1}.m^{-1} is the net metabolic demand calculated by initially subtracting the corresponding SMR from all MO_2 measurements and U is in m.s^{-1}. It corresponds to the Eq. (7) with a= 0.

2.6. Statistical analysis

Statistical analysis was carried out using R software and Graphpad prism. For each variable, normality and homoscedasticity were checked using Shapiro and Bartlett's tests, respectively. When normality and homoscedasticity were respected, differences between the exposure conditions were assessed using ANOVA one-way analysis of variance and Kruskal–Wallis one-way analysis of variance (KW) when it was not respected. For Fulton's condition factor K and Hepato-somatic index HSI Two-way ANOVA anlaysis was used. For all variables, Tuckey test was used as a post hoc test. For all tests, the results were considered to be significantly different when $p < 0.05$.

3. Results

3.1. Sediment analyses

3.1.1. Organic contaminants

In all sediments, the sum of PAHs concentrations was higher than those of PCBs and OCPs in which the concentrations were close to 0 ng.g^{-1} (Table 1). In detail, three PAHs were detected in the reference sediment, but at very low concentrations (i.e. naphtalene, fluorene, phenanthrene, Table S1). Overall, in SCOLA, the Dordogne sediment contained 5–10 times more PAHs than the Garonne sediment at T_0 and T_1, respectively. The compounds that were the most represented in Dordogne sediment were benzo (b+j + k) fluorantene, fluorantene, pyrene, chrysene+tripenylene, benzo-e-pyrene, and dibenzo (a,b+a,c) anthracene. The most present in Garonne sediment were benzo (b+j + k) fluorantene, fluorantene and pyrene. After 30 days of exposure (T_1), the PAHs concentration was reduced by 1.9 for the Dordogne sediment and by 3.6 for the Garonne sediment (Table S2). PCBs and OCPs concentrations were relatively low and constant over the 30 days of the exposure period (Table 1 and S1). The main PCBs found were CB52 and CB101 and the main OCPs was HCB (Table S1).

3.1.2. Metals

In the SCOLA, metal concentrations at T_0 were in the same range in Dordogne and Garonne sediments (Table 1, Table S2). The low metal concentrations contained in SCOLA reference came from the origin of the water (i.e. drilling water) and from low metal concentration in the reference sediment. At T_1, Dordogne metal concentrations in SCOLA were 1.2 times higher than for Garonne (Table 1). Zn, V, Cr, As, Pb, Co, Ni and Cu are the major metals found in Garonne and Dordogne SCOLA sediments (Table S2).

3.2. Water analysis

3.2.1. Quantification of pesticides

Very few pesticides were measured in the reference water, as all compounds were under the limit of quantification except carbendazime (Table 1, Table S3). While similar compounds were found in the waters of the Garonne and Dordogne rivers, much higher pesticide

concentrations were detected in the Garonne. Among these compounds, herbicides were the most abundant, with some insecticides also. Metabolites of acetochlore (ESA), metachlore (ESA, OA) and DEA are well represented in Garonne water. In water samples from Dordogne, high concentration of Propiconazole up to 1619 ng/L was measured (Table S3).

3.2.2. Quantification of Metals

Metal concentrations were measured in SCOLA water (Table 1, Table S4). The major metals found were As, Zn and Cu for the three conditions. Zn was more present in Reference than in Dordogne and Garonne waters.

V, Cr, Co, Ni, Ag, Cd, and Pb were found at low concentrations in all the conditions contrary to Cu, Zn and As which have higher concentration in Dordogne and Garonne water.

3.3. Fish tissues analyses

3.3.1. Organic contaminants

PAHs concentrations were not measured in tissues, because of the efficient metabolization of these compounds by fish (Kamman et al., 2017). PCBs and OCPs were systematically found at higher concentrations in liver samples than in muscle samples (Table 2, Table S5). There were 5–6 times more PCBs and 4 times more OCPs in the liver than in the muscles, whatever the time and type of exposure considered (Table 2). In general, the most concentrated congeners of PCBs were CB52, CB101, CB118, CB153, CB138 and CB180 in muscles and CB52, CB118, CB101 and CB153 in the liver (Table S5). Among PCBs, CB50, CB28, CB52, CB101, CB118, CB180 in muscles and CB28 in liver were more concentrated at T_0 than at T_1. During the one-month exposure, CB50 and CB101 were accumulated in the liver for all the conditions of exposure. CB138 and CB 180 were accumulated in the liver for Dordogne and Garonne conditions with higher concentrations for Dordogne. CB118 and CB153 concentrations in livers were increased only for the Dordogne condition. At T_1, no significant variation was observed in OCPs concentrations, regardless of exposure conditions, and 4–4′-DDE was the most prevalent. Similarly, PCBs concentrations in muscles remained almost constant. On the contrary, the total hepatic concentration of PCBs increased in fish exposed to the Dordogne river sediment, which was more contaminated than sediment from the Garonne river. The PCBs molecular pattern was dominated by penta- and hexachlorinated congeners.

3.3.2. Metal bioaccumulation

In gills, we observed a significant increase of Cr and Se at T_1 compared with T_0 (Table S5, ANOVA, $P < 0.05$). At T_1, Cr concentrations significantly increased for juveniles exposed to the Dordogne and Garonne conditions compared to the reference, and only in the Garonne condition for Se. On the contrary, we observed significant lower concentrations of Mn in the Garonne condition, compared to the Reference. At T_6, lower concentrations of Mn, Se and V were observed in the Garonne condition compared to the Dordogne, while As was more abundant in the Garonne condition. For Ag, Cd and Pb, values were often below detection limits.

In the liver, half of the metals (Co, Cu, Fe, Se, V and Zn) were significantly accumulated with time compared to T_0 (ANOVA, $P < 0.05$, Table 3, Table S5). At T_1, no specific differences were observed in the Garonne and Dordogne conditions compared to the reference, nor there was any difference between the two rivers. The same observations were made at T_6.

In muscles, metals were generally less accumulated than in the two other organs (Table 3, Table S5). We can nevertheless observe a decrease with time for tissue concentration of Cr, Cu, Mn, V and Zn with a significant difference between river conditions and the reference only for Se at T_1. On the contrary, at T_6, we observed an increase in As concentrations for the Dordogne and Garonne conditions compared to the reference.

3.4. Fulton's condition factor and Hepato-somatic index

The Fulton's condition factor K reflects the growth condition and the health status of fish. No significant difference was observed in K between T_0 and T_1 (1 month exposure to GGD conditions) whatever the considered exposure conditions. No significant difference was observed between these conditions at T_1, or after 5 months of depuration (Table 4; Tuckey Test, $p > 0.05$). However, K significantly decreased at T6 compared to T0 and T1 for each condition observed (Table 4; Tuckey Test, $p < 0.001$).

The Hepato-Somatic Index HSI reflects the healthy condition and also provides an indication on status of energy reserve in the fish. For this experimentation, HSI significantly decreased after one month of exposure (T_1) to Dordogne and Garonne conditions compared to Reference condition (Table 4; Tuckey Test, $p < 0.001$). After the 5-month depuration phase (T_6), there were no more differences among the 3 conditions.

3.5. AMR, SMR, AMS and U_{crit}

Metabolic rates (Fig. 1a) were estimated only at T_1 in fish exposed to reference, Dordogne or Garonne conditions. Mean values of AMR were similar between the 3 conditions, varying from 0.63 ± 0.1 for the Reference to 0.62 ± 0.23 and 0.63 ± 0.14 mg O2.g^{-1}.h^{-1} for Dordogne and Garonne conditions respectively. SMR tended to be higher in fish exposed to Dordogne (SMR_D=0.20 ± 0.05 mg O_2.kg^{-1}.h^{-1}) and Garonne conditions (SMR_G=0.22 ± 0.15 mg O_2.kg^{-1}.h^{-1}) than in the individuals exposed to the Reference condition (SMR_R=0.14 ± 0.06 mg O_2.kg^{-1}.h^{-1}). However, statistical analysis did not reveal any significant difference between exposure conditions whatever the metabolic rate considered: SMR (KW test: $X^2 = 3.62$, df = 2, $p = 0.16$), AMR (KW test: $X^2 = 1.29$, df = 2, $p = 0.52$) or AMS (KW test: $X^2 = 4.06$, df = 2, $p = 0.13$).

3.6. Swimming efficiency

For the metabolic variables, U_{crit} was estimated only at T_1 in fish exposed to Reference, Dordogne and Garonne conditions. U_{crit} for the Reference (6.26 ± 1.85 BL.s^{-1}) was higher to U_{crit} measured for Dordogne condition (5.78 ± 0.99 BL.s^{-1}) and Garonne condition (5.50 ± 0.67 BL.s^{-1}) but did not significantly differ according to the type of exposure (KW test: $X^2 = 2.29$, df = 2, $p = 0.31$).

For each exposure condition, a relationship between oxygen consumption (COT_{net} in mg O_2.h^{-1}.kg^{-1}) and swimming speed (m.s^{-1}) was fitted by the least square method using Eq. (6). The values of the corresponding constants and R^2 are reported in the supplementary (Table S6). The effect of the condition of exposure on the relationship between swimming speed (U) and COT_{net} was examined using Eq. (9) (Fig. 1b). Optimal swimming speeds (U_{opt}) and COT_{net} at U_{opt} were also calculated and reported on Fig. 1b. COT_{net} increased with the swimming speed. The effect of exposure conditions was examined through the relationship between swimming speed (U) and COT_{net}. $COT_{net,}$ increased with the swimming speed, but the cost of swimming at a given speed was not significantly influenced by the exposure condition. Specifically, the optimal swimming speed (U_{opt}) and COT_{net} at U_{opt} were also calculated for each exposure conditions and reported on Fig. 1b, suggesting that dissimilarities may exist.

3.7. Liver histopathology

Liver sections were observed microscopically and a semi-quantification using a 5-grade scale was performed to document the intensity of lipid and glycogen storage into hepatocytes (Fig. 2). At T_1, all juveniles exposed to the reference condition displayed a generalized and marked storage of lipids and glycogen into hepatocytes, appearing

Table 4
European sturgeon juvenile biometrics, Fulton's condition factor K and Hepato-somatic index HSI. TL1 was measured from the mouth to the end of the tail. Means±SE.

Time of exposure	Treatment	Number of fish	Total weight (g)	Gutted weight (g)	Total Length (TL1, mm)	K	HSI
T_0	Reference	n = 8	11.28 ± 2.49	9.66 ± 2.11	132.00 ± 9.27	0.48 ± 0.02[a]	2.47 ± 0.34[a]
T_1	Reference	n = 20	20.65 ± 4.81	17.89 ± 4.28	162.75 ± 10.86	0.47 ± 0.04[a]	1.99 ± 0.35[b]
	Garonne	n = 20	19.73 ± 6.65	17.38 ± 5.92	160.50 ± 16.21	0.46 ± 0.03[a]	1.49 ± 0.31[c]
	Dordogne	n = 20	17.85 ± 4.57	15.50 ± 4.08	156.75 ± 13.18	0.46 ± 0.03[a]	1.37 ± 0.35[c]
T_6	Reference	n = 10	88.96 ± 27.97	78.81 ± 25.58	293.40 ± 29.78	0.34 ± 0.02[b]	1.42 ± 0.23[c]
	Garonne	n = 10	75.31 ± 22.33	66.39 ± 20.28	271.40 ± 35.37	0.38 ± 0.10[b]	1.43 ± 0.34[c]
	Dordogne	n = 10	86.06 ± 34.63	76.98 ± 32.03	289.10 ± 38.15	0.34 ± 0.03[b]	1.62 ± 0.35[b,c]

A total of 98 fish was sampled, number of fish per treatment is indicated in bracket. Different letters indicate significant differences between time and conditions (two-way ANOVA, $p < 0.05$).

as large vacuolated with droplets or with a clear granular abundant cytoplasm. The mean score was 4.0 ± 0.0 in the reference group that is significantly different from Dordogne and Garonne conditions, displaying respectively a mean score of 2.3 ± 1.3 and 2.0 ± 1.2 (Kruskal-Wallis, $p < 0.0001$). Juveniles exposed to Dordogne and Garonne conditions displayed a lipid depletion compared to fish from Reference condition. This means that fish from Reference condition have bigger liver and higher lipids and glycogen at T_1. A higher granulomas frequency was also observed in the liver of fish exposed to the Garonne and Dordogne conditions. Some foci of infiltration by inflammatory cells were also observed in fish with no difference in incidence between conditions (incidences being respectively 7/10; 6/10 and 8/10 in Dordogne, Garonne and reference condition).

At T_6, hepatocytic storage was minimal in certain fish with no difference between groups. Foci of hepatic necrosis were only observed in liver of fish from the Garonne condition (n = 6/10) and a group effect was demonstrated ($p < 0.001$) and could result from parasitic migration into the liver. Additionally, two fish (out of 10) of the Dordogne condition displayed trematodes in bile ducts with no parenchyma lesions.

4. Discussion

This study aimed to evaluate the consequences of young *A. sturio* being exposed to the environmental conditions of release sites selected from current conservation programs within the GGD catchment. Overall, aerobic metabolism, swimming efficiency, and associated energy costs, Fulton's Condition factor were not impaired by different conditions, which is contrary to Hepatosomatic Index. K and HSI also decreased with time of exposure. Compared to the Reference condition, Garonne and Dordogne conditions tended to induce a moderate inflammatory response in the liver associated with a significant lower.

Organic pollutants and excess metals found in the GGD basin are mainly due to human activity (e.g. agriculture, industry; Maury-Brachet et al., 2008). The implication of pollutants in the European sturgeon disappearance has been mentioned even if never demonstrated. Based on previous environmental analysis evaluating the quality of different spawning grounds (Delage, 2015), 2 sites, Pessac-sur-Dordogne in Dordogne River and La Réole in Garonne River, were selected for deeper investigation.

Sediment was the focus of the study because of the ecology of sturgeons, notably in terms of reproduction and foraging. Sturgeon reproduction occurs in rivers close to the substrate. During this period, fertilized eggs become adhesive and grip onto any available substrate e.g. aquatic plants, rocks, coarse sediment. At larval stage, young sturgeons live in direct contact with sediment, eating insect larvae such as chironoms from the sediment. Sturgeon is a bottom-dwelling fish which lives near the sediment/water interface. Secondly, pollutants found in the Dordogne and Garonne rivers are mainly of the hydrophobic type (PAHs, PCBs, OCPs) and thus accumulated in sediment. Chemical analyses of environmental sediment revealed that Garonne contains a higher organic pollutant diversity (PAHs, PCBs, OCPs) and globally higher concentrations than Dordogne. Over the experimental exposure period, PAHs concentration in sediment decreased with time whereas the PCBs and OCPs concentrations were relatively low and constant. In contrast, Dordogne sediments were more contaminated by metals, mainly represented by Zn, Cu, Cr, Pb, Ni, As, Co. Metals concentrations globally increased with the time of exposure.

PAHs, PCBs and OCPs concentrations were not measured in water. Due to their hydrophobic properties, these compounds are adsorbed on particulate matter and then accumulated in sediment. Chemical analyses of water focused on water soluble compounds such as metals and herbicides. Cu and Zn contaminations come from fungicide treatments used in vineyard of GGD basin and their concentrations can vary according to their use in vineyards throughout the year. Similar pesticide profiles were found in the two rivers with higher concentrations of pesticides detected in Garonne. When we compared the measured pollutant concentrations with the environmental quality standards (EQSs) for priority substances in surface water (directive 2008/105/EC), only Cu concentration was higher than the EQSs in both rivers and in SCOLA waters. This means that this metal represents a potential risk for fish living in Dordogne and Garonne, especially in Dordogne.

In addition, it can be stated that the current level of pollution in Garonne and Dordogne is moderate since most of organic pollutants and metals concentrations measured were below environmental quality standard (EQSs).

Water samplings in Garonne and Dordogne were performed in October-November. Concentrations of pollutants such as pesticides can vary according to the season. Our experiment mimic environmental conditions in autumn i.e. at the period were juveniles of sturgeon are released in Garonne and Dordogne rivers. To overcome the potential degradation of the most labile organic pollutants, 1 m^3 of water in each SCOLA (1/4 of SCOLA volume) was renewed every week and water samplings in Dordogne and Garonne were performed every week.

To confirm contamination following exposure in SCOLA, pollutant bioaccumulation was also investigated. POP analysis of fish tissues indicated very low bioaccumulation of PCBs and OCPs: between T_0 and T_1 we observed only a 50% increase of PCBs content in the liver of fish from the Dordogne condition, and 7% for Garonne. The higher PCBs and OCPs concentrations in the liver compared to the muscles could be explained by the higher lipid content in the liver (37 ± 16% in liver versus 9 ± 3% in muscles). This has been observed in other fish species such as meagre, seabass, mullet, flounder and sole (Bodin et al., 2014). PCBs levels in European sturgeon were lower than the concentrations measured in other fish species from the same area (Bodin et al., 2014). In our experiment, PCBs accumulated in liver of fish can result from uptake from the dissolved phase (not measured), the suspended matter and the sediment. It is worth noting that contamination by food is unlikely, because all conditions received the same food, and PCBs levels remained stable between T_0 and T_1 in fish from the reference group.

For metals, Cu and Zn concentrations measured in gills and muscles were similar to those reported in *A baeri* caught in the Gironde estuary. Cd was not detected in muscles of *A baeri* nor *A sturio*. Pb concentration in muscles was around 30 times higher for *A Baeri* compared to *A sturio*. In the liver, these 4 metals (Cu, Zn, Cd, Pb) reached higher concentrations in *A baeri* than in *A. sturio*. Differences in metal levels between these two species can be explained by different times and means of

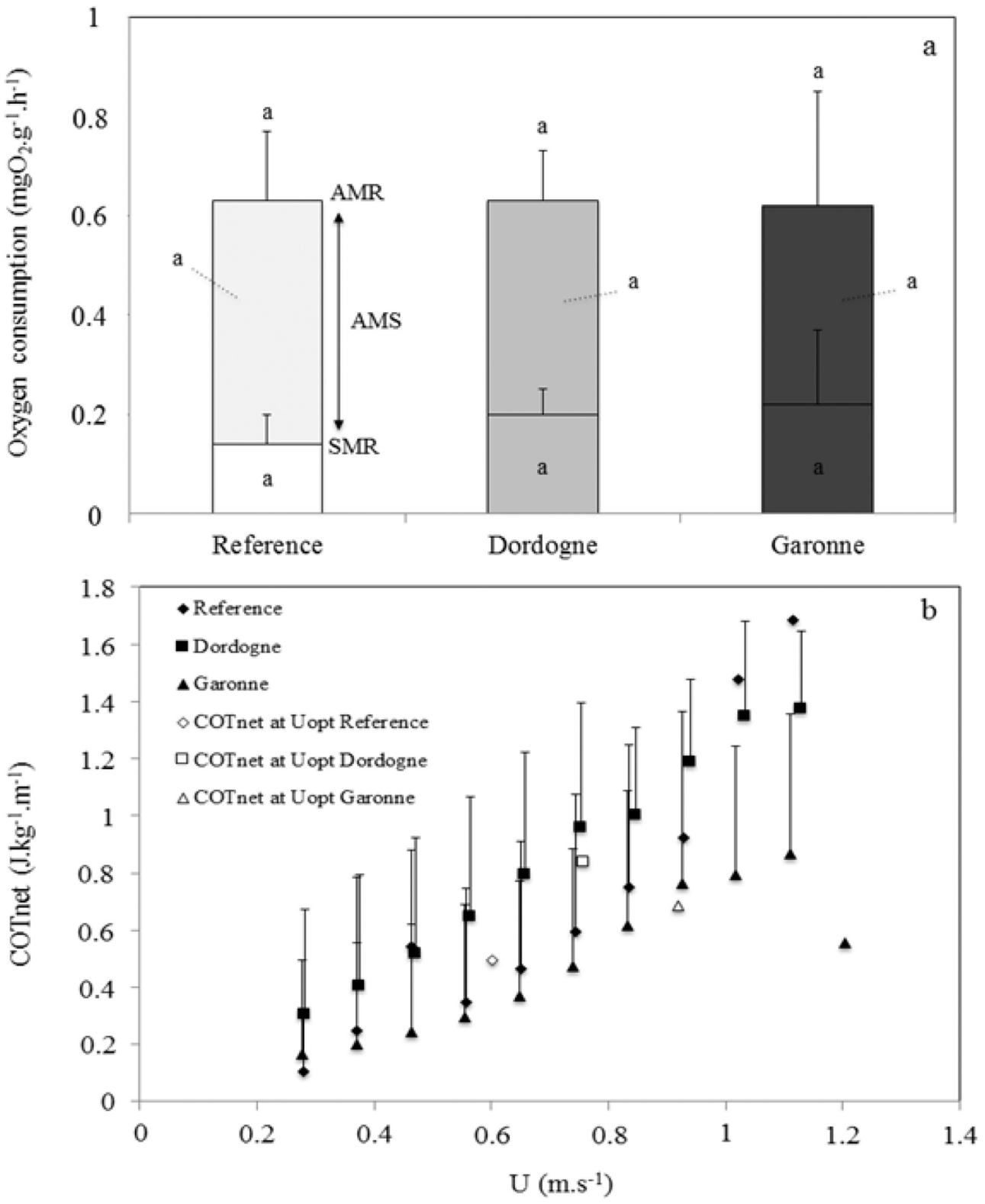

Fig. 1. a) Active metabolic rate (AMR), standard metabolic rate (SMR) and aerobic metabolic scope (AMS) b) relationship between net costs of transports (COT_{net}) and swimming speeds (U) for European sturgeon juveniles exposed for one month to Reference, Dordogne conditions or Garonne conditions (noted R, D and G). White symbol represents the COT_{net} at the optimal swimming speed (U_{opt}). Number of fish tested per condition of exposure: $n_R = 7$, $n_D = 11$, $n_G = 12$. All the results are expressed as mean values ± SD. Different letters above bars indicate a significant difference between conditions (ANOVA, $p < 0.05$).

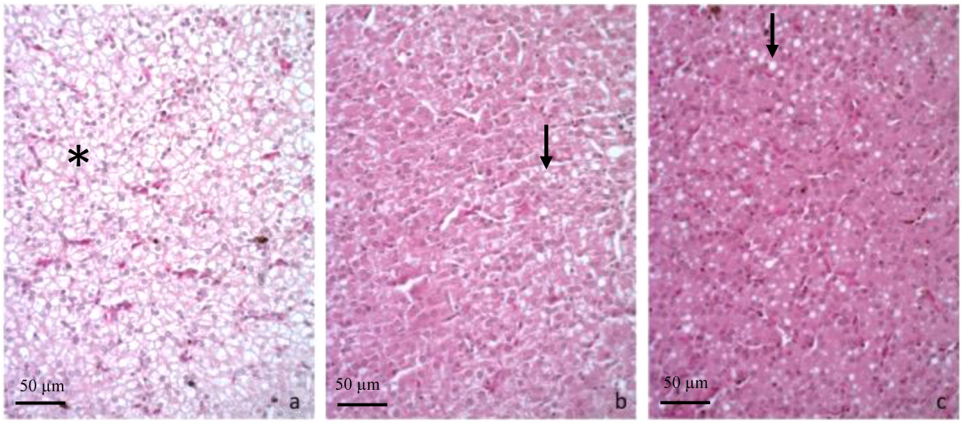

Fig. 2. Hepatocytes from fish observed at T_1, after one month of exposure to the experimental condition of a) Reference, b) Garonne and c) Dordogne. n = 10 per condition. Reference is significantly different from Dordogne and Garonne (Kruskal-Wallis, $p < 0.0001$). See the global fainted / pale staining (* in a) compared to the diffuse eosinophilic staining with only a few vacuolated hepatocytes (arrow in b and c).

exposure. *A. baeri* were contaminated by chronic exposure through both water and food, whereas in our study, *A sturio* was exposed to chemicals through the water column and sediments only for one month. Metal contamination of juveniles in the Dordogne and Garonne experimental conditions was organ dependent, with major bioaccumulation in gills and liver. The metal profile in tissue corresponds to those mainly measured in sediment and water (for Zn, Cu, As, V and Co). No link can be established between metal content in food and in fish tissues: higher concentrations of Ni, Pb and As were observed in chironoms than in fish. On the contrary Cr and Se were more elevated in fish than in chironoms. With recognized contamination by metals and low bioaccumulation of PCBs and OCPs, the risk for juvenile sturgeon is moderate.

To provide information on potential pollution impacts on the physiological status of young sturgeons, two condition indices were first measured in the present study: the Fulton's condition factor K and the Hepatosomatic Index HSI. K is an estimation of the growth condition, and depends on the type of growth i.e. isometric or allometric. Fish with a high K value are relatively heavy for their length, while fish with a low K value are light for their length. Based on this statement, decrease of K value over time for each condition may reflect an important increase in length compared to weight. K value may also vary according to sex, gonad development or season (reference). K decreased at T_6 for each condition tested. This time variation was not linked with our conditions of exposure, since K vary similarly among these 3 conditions tested. In addition, decrease of K was not due to loss of weight since fish gained weight in time (Reference: from 14.4 to 130.1 g, Dordogne from 11.5 to 149.8 g, Garonne from 12.8 to 110.4 g). On the other hand, HSI gives an indication of the size and the health status of the liver, which is particularly important for both energy reserves and process of detoxification. HSI is therefore often used as an estimate of the energy status of fish (Chellappa et al., 1995). HSI was significantly lower for the Garonne and Dordogne conditions compared to the Reference after one-month exposure. Lower HSI reflects a smaller liver relative to the body. Our results also show that fish at T_0 had bigger livers and higher lipids and liver glycogen contents than at T_1 whatever the considered conditions. Decrease of HSI over time may be due greater growth in terms of length than weight. During the experimental period (autumn and winter) fish may use their glycogen reserves as fuel for growth and survival, which will deplete their glycogen stores. In addition, reduction of HSI of fish from the Garonne and Dordogne conditions compared to the Reference after one-month exposure was consistent with liver histopathology analyses. This showed that juveniles exposed to Dordogne and Garonne conditions displayed a lipid depletion compared to fish from the Reference condition. Two hypotheses are possible: (1) fish from the reference conditions ate more, (2) fish from the Garonne and Dordogne conditions consumed more reserve than fish from Reference condition in order to maintain their homeostasis and use detoxification processes. Furthermore, a higher granulomas frequency was observed in the liver of fish exposed to Garonne and Dordogne conditions. Granulomas are generally formed during inflammation in response to accumulation of foreign substances in the liver. Exposure to metals and POPs from Dordogne and Garonne rivers could have impaired the liver structure by generating reactive oxygen species (ROS) resulting in oxidative stress, cell death and tissue inflammation (Rahbar et al., 2020).

Other biological parameters were assessed, e.g. aerobic metabolism, swimming performance, and the related cost of swimming. To our knowledge, aerobic metabolism has never been measured in European sturgeons, but has been in other Acipenseridae. The metabolic rates measured (in average SMR=0.2 $mgO_2.g^{-1}.h^{-1}$; AMR= 0.65 $mgO_2.g^{-1}.h^{-1}$; AMS=0,44 $mgO_2.g^{-1}.h^{-1}$) were in the range of those estimated in Green sturgeon (Langford et al., 2005), Adriatic sturgeon (McKenzie et al., 2001) and White sturgeon (Crocker and Cech, 1997). The slight effect of treatment on general metabolism was consistent with the results of U_{crit}, meaning that juvenile sturgeons have similar swimming capacity whatever the exposure conditions when challenged with a swimming step protocol. Few measurements of swimming performance of other sturgeon species have already been published, but not in the context of investigation pollutant impact. In addition, U_{crit} measured on white sturgeon (Counihan and Frost, 1999), Shovelnose sturgeon (Adams et al., 2003) and Green sturgeon (Langford et al., 2005) were 1.5–2 times lower than those measured in the present study. Only the U_{crit} reported by Hoover et al. (2011) (U_{crit}=95,2 $cm.s^{-1}$) in Shovelnose sturgeon is in the range of the one of this present study. Differences in species and ages of fish are likely to explain the differences observed in U_{crit}. The optimal swimming speed U_{opt} was also estimated and represents the swimming speed for which cost of transport is minimal (Videler, 1993). While U_{opt} is higher in fish exposed to Garonne conditions than in fish exposed to Dordogne and the Reference, these results are not significant. This present study also suggests that the net costs of swimming for *A. sturio* juveniles at a given swimming speed do not change between conditions. These results mean that fish released in Dordogne or Garonne Rivers will have similar capacity to adapt to and to explore their habitat for food resources or for migration. The swimming performance and associated costs could be completed by the analysis of the anaerobic part of the metabolism required for U_{crit}. During intense effort such as swimming, fish could be exposed to a prolonged period of oxygen deprivation and accumulate an oxygen debt that is repaid during recovery by a substantial increase in oxygen consumption. Oxygen debt can be therefore relevant to be estimated after the swimming challenge.

On the other hand, because of their different life history traits and habitats, juveniles of sturgeon face high variations in temperatures and oxygen levels in the GGD basin, depending on the location and season

(Travade and Carry, 2008). In a context of global change, an increase of hypoxic events and warmer temperatures are expected in the GGD basin especially in summer. The fate of pollutants and organism responses are dependent on the temperature of the environment. In the present study, environmental variations of parameters such as increase of temperature or hypoxia events were not simulated, which means our experiment did not totally mimic the natural conditions of Dordogne and Garonne Rivers. High amplitude of variations of physico-chemical parameters can also represent an additional stress to aquatic organism in particular to early life stages of European sturgeon (Delage et al., 2014).

5. Conclusion

Regarding the pollutant accumulation in tissues and their physiological and histological responses, it can be assumed that current contamination in the GGD catchment is not likely to adversely affect European sturgeon juveniles in current water temperature and oxygen conditions. In view of the low effect observed on fish health, we can consider that European sturgeon juveniles are tolerant enough to have adapted to the life conditions of Garonne and Dordogne rivers. However, it remains essential to carry out further molecular and physiological analyses to have a more holistic view, in a wider variety of environmental conditions.

This work is confirmed by the recent study of Acolas et al. (2020) that described for the first time chemical the impregnation levels and health status of European sturgeons in the natural environment (Gironde estuary). From blood samples collected since 2007 on wild juveniles of *A. sturio*, (Acolas et al., 2020) reported low inorganic and organic contamination in European sturgeon from Gironde estuary. Environmental conditions may vary in the next years and it will be necessary to reduce environmental pressure on GGD basin in order to guarantee a high survival rate during release of juveniles in GGD basin and to sustain the European sturgeon population.

Overall our data make it possible to estimate the current physiological conditions of European sturgeon and their capacity to sustain the GDD environment. The data obtained are also of fundamental importance, because, through their integration in modelling approaches, they may contribute to test scenarii to predict the dynamics of the last European sturgeon population in light of their changing habitat.

CRediT authorship contribution statement

J. Lucas: Investigation, Methodology Validation, Writing – original draft. **C. Lefrancois:** Investigation, Methodology, Writing – original draft, Writing – review & editing. **C. Gesset:** Resources, Investigation. **H Budzinski:** Methodology, Validation, Supervision, Writing – review & editing. **P. Labadie:** Methodology, Validation, Writing – review & editing. **M. Baudrimont:** Methodology, Investigation, Validation, Writing – review & editing. **A. Coynel:** Methodology, Investigation, Validation, Writing – review & editing. **K. Le Menach:** Methodology, Investigation, Validation, Writing – review & editing. **P. Pardon:** Resources, Investigation. **L. Peluhet:** Resources, Investigation. **N. Tapie:** Resources, Investigation. **P. Lambert:** Methodology, Software, Formal analysis. **T. larcher:** Methodology, Investigation, Writing – review & editing. **E. Rochard:** Conceptualization, Investigation, Writing – review & editing. **P. Gonzalez:** Conceptualization, Investigation, Writing – review & editing. **J. cachot:** Conceptualization, Validation, Supervision, Project administration, Funding acuiqisition, writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112720.

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