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1 **Effects of pyrolytic and petrogenic polycyclic aromatic hydrocarbons on**
2 **swimming and metabolic performance of zebrafish contaminated by**
3 **ingestion**

4
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25

26 **Abstract**

27

28 Depending on their origins, polycyclic aromatic hydrocarbons (PAH) are characterized by
29 different chemical properties. Petrogenic PAH (e.g. from fossil fuels) and pyrolytic PAH (e.g.
30 those produced by incineration processes) are therefore expected to affect organisms
31 differently. The impact of trophic exposure to these PAH was investigated on swimming and
32 metabolic performance of zebrafish *Danio rerio*. Two-month-old juveniles and six-month-old
33 adults were individually challenged following a swimming step protocol. While pyrolytic
34 exposure did not affect fish whatever the duration of exposure, it appeared that petrogenic
35 PAH impaired adults' performance. Indeed, the active metabolic rate in petrogenic PAH-
36 contaminated adults was significantly reduced by 35%, and critical swimming speed by
37 26.5%. This was associated with cardiac abnormalities, which are expected to contribute to
38 the reduction of oxygen transport, particularly during intensive effort. These results may be
39 due to the different composition and toxicity of PAH mixtures.

40

41 *Keywords:* trophic exposition, petroleum hydrocarbons, critical swimming speed, aerobic
42 metabolic rate, sub-lethal concentration

43

44 1. Introduction

45

46 Locomotion performance in fish is considered to be a relevant biological function
47 contributing to survival (e.g. Graham et al., 1990; Jones et al., 1974; Plaut, 2001; Stobutzki
48 and Bellwood, 1994; Swanson et al., 1998), since it is related to predator–prey interactions,
49 food research and migration (Drucker and Jensen, 1996; Hammer, 1995; Plaut, 2001; Reidy et
50 al., 1995; Videler, 1993; Walker et al., 2005; Watkins, 1996;). Critical swimming speed (i.e.
51 the maximal velocity a fish can reach during a swimming step protocol, U_{crit} ; Hammer, 1995)
52 is frequently employed as an indicator of swimming capacities in a varying environment (e.g.
53 Beamish, 1978). Since U_{crit} notably depends on the maximal ability of fish to provide energy
54 during sustained swimming activity, these studies are often associated with the assessment of
55 metabolic performance, which contribute to a better view of the environmental impact.
56 Aerobic metabolic scope (AMS; Brett, 1964; Fry, 1947, 1971) is defined as the difference
57 between (i) the active metabolic rate (AMR), which is the highest metabolic rate the organism
58 can sustain under maximal activity and (ii) the standard metabolic rate (SMR), the metabolic
59 rate necessary to maintain vital functions and measured under resting conditions. Metabolic
60 and swimming performance are known to be modulated by a set of environmental parameters,
61 such as temperature (Blier et al., 1997; Farrell, 2007; Rome et al., 2007), dissolved oxygen
62 (Diaz, 2001; Diaz et al., 2004) or pollutants (Johansen and Jones, 2011; Lefrançois and
63 Claireaux, 2003; Marit and Weber, 2012; Shingles et al., 2001; Schurmann and Steffensen,
64 1997; Thomas et al., 2013).

65 This study aimed at investigating responses of fish exposed to environmentally relevant
66 concentrations of PAH through the assessment of AMS and U_{crit} as indicators of the
67 physiological state of the organism (Fry, 1947). The increase of anthropogenic activities in
68 coastal areas induces discharges of PAH in aquatic ecosystems. Due to their high

69 liposolubility, PAH are typically adsorbed by organic matter or marine sediments,
70 bioaccumulated by organisms at the lowest trophic levels (e.g. invertebrates; Bustamante et al.,
71 2012; O'Connor and Lauenstein, 2006) and transferred through trophic chains (Hylland, 2006;
72 Vignet et al., 2014). Past studies on fish have demonstrated that hydrocarbon exposure causes
73 a decrease of U_{crit} . For instance, *Danio rerio* showed a 15% and 22% reduction of U_{crit} while
74 exposed to crude oil (Hicken et al., 2011) and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin;
75 Marit and Weber, 2012), respectively. Moreover, PAH could induce damage of tissues and/or
76 organs involved in oxygen transport (e.g. fish gills, Fasulo et al., 2012; mussel gills, Cappello
77 et al., 2013). This could induce a decrease of AMR as observed by Davoodi and Claireaux
78 (2007) after acute PAH exposure of *Solea solea*. In addition, the implementation of
79 detoxification processes which are energy costly have already been demonstrated. These
80 processes are likely to increase costs of maintenance and, consequently, SMR. The
81 consequent potential reduction of AMS would reflect a decrease in the capacity of fish to
82 sustain energy-demanding activities.

83 The effects of PAH mixtures were tested on zebrafish *Danio rerio* (F. Hamilton, 1822)
84 contaminated by ingestion. Both pyrolytic PAH (PY-PAH, resulting from the combustion of
85 organic matter) and petrogenic PAH (HO-PAH, originated from fossil fuels; Hylland, 2006)
86 were considered in the present study. Present as complex mixtures in the environment, PY-
87 PAH and HO-PAH show different chemical properties and may induce various effects on
88 aquatic life. The main hypothesis was that chronic exposure to PY-PAH and HO-PAH would
89 impair AMS by increasing SMR and/or reducing AMR. The consequent potential reduction of
90 AMS would indicate a decrease in the capacity of the fish to support oxygen-demanding
91 activities beyond SMR and consequently a reduction of U_{crit} . In addition, PAH may induce
92 anatomic impairments due to teratogenic effects (Hawkins et al., 1990; Myers et al., 1991).
93 Swimming and metabolic functions were explored, as well as the cardiac and skeletal muscle

94 structures, as two key tissues involved the tested functions.

95

96 **2. Materials and methods**

97

98 *2.1. General production of zebrafish*

99

100 Pairs of zebrafish (wild-type Tuebingen strain, TU) were reared together in 10 L tanks in the
101 laboratory. Aquaria were filled with a mix of osmosis and filtered tap water which was
102 characterized by the following parameters: temperature 28 ± 0.5 °C, conductivity 300 ± 50
103 $\mu\text{S}\cdot\text{cm}^{-1}$, oxygen saturation $\geq 80\%$, pH 7.5 ± 0.5 . Fish were reared with a photoperiod of 14 h
104 light/10 h dark. Ammonia, nitrite and nitrate remained within recommended ranges (Lawrence,
105 2007). The fish were fed twice daily with uncontaminated commercial dry food (INICIO Plus,
106 BioMar, France) and once by *Artemia* sp. nauplii (Ocean Nutrition, Belgium), occasionally
107 supplemented with red sludge worms (Boschetto, Poland).

108 Over a period of one month, spawn was obtained from pairs weekly following the protocol
109 described in Lucas et al. (2014a) and Vignet et al. (2014). Briefly, the day before spawn was
110 collected, these fish couples were isolated in 1 L breeding tanks (Aqua Schwarz, Germany).
111 After collection, fertilized and normally developed eggs were selected using a binocular
112 microscope. Spawn was then mixed to avoid any parental influence. From 2 weeks onwards,
113 fish were kept in groups of 30 individuals in 10 L aquaria in the same specific rearing system
114 described above.

115

116 *2.2. Food contamination protocol*

117

118 Artificial dry food (INICIO Plus, BioMar France) was spiked with mixtures of PAH as

119 described in detail by Vignet et al. (2014). Briefly, the pyrolytic fraction (*PY*) was extracted
120 from natural sediment sampled at the site of Oissel (Seine Bay, France), while the petrogenic
121 fraction (*HO*) was extracted from heavy Erika fuel oil. For both types of *PY* and *HO* mixtures,
122 quantification of the 16 individual PAH of the US EPA list was made, according to a protocol
123 described in Budzinski et al. (1997) and Cachot et al. (2007). The food was then spiked with
124 the extracted *PY* or *HO* fractions according to a protocol adapted from Vicquelin et al. (2011).
125 The target concentration was 15000 ng.g⁻¹ of food and based on the sum of the 16 PAH from
126 the US EPA list in dry weight. This corresponds to three times the PAH concentration
127 organisms may be exposed to in their natural environment (Cachot et al., 2006; Cailleaud et
128 al., 2007; Payne et al., 2008). This high concentration was used to highlight the toxicological
129 effects on the physiological performances investigated in this study. It is called *X* in the rest of
130 the manuscript. Food size was adapted to the developmental stage of *D. rerio*. A *control*
131 treatment was also considered, and consisted of feeding groups of fish with uncontaminated
132 food, treated only by the solvent dichloromethane. This solvent was used to improve PAH
133 incorporation into the food, and then evaporated. PAH compositions and concentrations in the
134 diet are described in detail in Table 1.

135

Table 1. Concentration of 21 US-EPA PAH in contaminated dry food. Values are expressed as mean±standard deviation. n.d.=not detected

PAHs	Molecular weight (g.mol ⁻¹)	Concentration in dry food (ng.g ⁻¹)			
		pyrolytic PAH		petrogenic PAH	
		Solvent	3X	Solvent	3X
Naphtalene	128.2	4.01±2.21	199.13±32.06	3.79±0.97	405.02±73.41
Acenaphthylene	152.2	0.87±0.26	124.82±12.38	1.31±0.51	13.10±12.77
Acenaphthene	154.2	1.91±0.64	99.49±10.80	27.29±26.07	190.11±17.26
Fluorene	166.2	2.62±0.48	148.22±18.02	2.34±1.05	312.01±25.07
Dibenzothiophenes	184.26	0.68±0.46	117.95±10.61	0.34±0.16	545.94±53.56
Phenanthrene	178.2	9.36±3.73	1029.78±88.76	6.11±2.73	1278.88±51.23
Antracene	178.2	0.78±0.31	598.14±53.81	0.37±0.22	219.76±9.75
Fluoranthenes	202.3	1.37±0.39	1968±179.78	1.92±0.71	145.08±17.70
Pyrenes	202.3	1.62±0.21	1648.54±179.45	1.45±0.27	709.13±64.10
Benzo[a]anthracene	228.3	0.76±0.26	2286.28±200.96	n.d.	542.78±29.08
Chrysene+Triphenylene	228.3	0.77±0.26	2984.46±240.64	n.d.	1073.29±78.69
Benzonaphthothiophene	252.3	6.53±0.73	660.11±56.98	n.d.	572.96±35.67
Benzo[b,k,j]fluoranthenes	252.3	1.54±0.56	3164.65±269.51	n.d.	362.75±17.62
Benzo[e]pyrene	252.3	0.89±0.37	1267.09±108.51	n.d.	535.54±25.67
Benzo[a]pyrene	252.3	0.72±0.55	1395.49±146.63	n.d.	342.12±10.42
Perylene	252.3	0.96±0.48	437.37±39.40	n.d.	172.01±13.89
Indeno[1,2,3-cd]pyrene	276.3	n.d.	1218.26±123.95	n.d.	68.42±10.88
Dibenzo[a]anthracene + Dibenzo[a]chrysene	276.3	2.22±1.74	369.38±53.74	n.d.	112.55±6.26
Benzo[g,h,i]perylene	276.3	n.d.	913.77±88.29	n.d.	480.53±12.21
Sum PAHs		34 ± 6	17305 ± 4798	39 ± 30	8082.5 ± 305

136

137

138 2.3. Zebrafish PAH exposure protocol

139

140 Contamination through food was conducted by feeding zebrafish larvae twice a day with one
 141 of the treatments (i.e. *control* and *X* for both types of contamination *PY* and *HO*). The diet
 142 started from the first meal (5 days post fertilization, dpf) until the age at the test: 2 months and
 143 6 months for juveniles and adults, respectively. In accordance with the protocols in Vignet et
 144 al. (2014), larvae were fed ad libitum and then, starting from 2 months old, the ration of food
 145 was decreased by 5% to 2% of the biomass in each tank in order to maintain constant growth.
 146 For all fish, brine shrimp were distributed as complementary food once a day.

147 *PY* exposure took place during the second semester of 2011 and *HO* exposure was carried out
 148 in summer 2013. Quantification of hydroxylated metabolites in larvae at 15 dpf indicated a
 149 dose-dependent increase of metabolites, confirming successful contamination (total

150 concentrations of hydroxylated metabolites for *PY*-PAH: *control* = 9.1 ng.g⁻¹ of tissue, *X* =
151 275 ng.g⁻¹; Vignet et al., 2014).

152 For the *X* treatment tested, the average concentrations found were 136 ng.g⁻¹ for *PY* larvae
153 and 42 ng.g⁻¹ for *HO* larvae, while it was 2 or 4 ng.g⁻¹ in the *PY* and *HO control* fish,
154 respectively (Vignet et al., 2014). It appears that PAH concentration is higher for *PY* than for
155 *HO*.

156

157 *2.4. Aerobic metabolic scope and critical swimming speed*

158

159 *2.4.1. Experimental set-up*

160 For both types of contamination (i.e. *PY*- and *HO-PAH*), two identical 170 mL swimming
161 respirometers (Loligo Systems, Denmark) were used to assess the swimming and metabolic
162 performance of fish. Each swimming respirometer was composed of (a) a swimming chamber,
163 where the fish was placed to be tested, (b) a motor fitted with a three-bladed propeller to
164 control water flow and (c) honeycomb placed at each side of the swimming chamber to
165 laminarize the water flow. Each swimming respirometer was submerged in a 20 L buffer tank,
166 and filled with temperature-controlled (i.e. 28 °C) and oxygenated mixed water as in the
167 rearing system. The oxygen consumption (MO₂ in mg O₂.g⁻¹.h⁻¹) associated with the activity
168 of fish was measured by intermittent-flow respirometry (Steffensen, 1989). Water supply in
169 each swimming respirometer was provided by flush pumps, which controlled water flow from
170 the buffer tank to the swimming respirometer. This allowed alternation between phases of
171 oxygen renewal and phases of MO₂ measurement with a cycle of 5:20 min. In addition, a
172 peristaltic pump was used to create a continuous water flow from the respirometer to the
173 oxygen probe (flow through an oxygen minisensor, PreSens, Germany). The probe was
174 connected to an oxymeter (Microx, PreSens, Germany) to record the level of dissolved

175 oxygen in the water every 5 s. The probe was calibrated once at the beginning of the swim test
176 using 0% and 100% air saturation for a controlled temperature of 28 °C.

177

178 2.4.2. *Experimental protocol*

179 The fish were starved 24 h before being transferred individually to a swimming respirometer.
180 They were then individually submitted to a swimming challenge. Each experimental trial
181 consisted of challenging two fish in parallel exposed to one of three treatments: (i) fish fed by
182 the *control* food and (ii) fish exposed to *PY-* or *HO-PAH*. Each individual was submitted to a
183 short acclimation swimming period (~5 min) during which the velocity was increased to 2
184 body lengths per second ($\text{BL}\cdot\text{s}^{-1}$). Then, fish were left undisturbed with a water flow of 0.5
185 $\text{BL}\cdot\text{s}^{-1}$ for 2 h to permit recovery from handling stress. The swimming challenge started by
186 increasing water flow by steps of $1.5 \text{ BL}\cdot\text{s}^{-1}$ from 0.5 to $6.5 \text{ BL}\cdot\text{s}^{-1}$ and steps of $0.75 \text{ BL}\cdot\text{s}^{-1}$ to
187 the end of the test. Each step lasted 20 min, during which fish MO_2 was measured. The
188 experiment stopped when the fish fatigued, i.e. when it did not manage to swim against the
189 current and stay on the honeycomb. Speed was then decreased to $0.5 \text{ BL}\cdot\text{s}^{-1}$ for a recovery
190 period of 1.5 h. Fish were then removed from the swimming respirometer and anaesthetized
191 using benzocaine ($50 \text{ mg}\cdot\text{L}^{-1}$). Standard and total length, mass and sex of each individual
192 were determined; characteristics of the fish tested in the swim tunnel are described in Table 2.
193 Before and after each trial, a blank measurement was performed to quantify microbial oxygen
194 consumption in the swimming respirometer. The average of these two values was subtracted
195 from the measured oxygen consumption. Each fish was tested once.

Table 2. Biometry of fish for each PAHs fractions tested (Mean±SE). SL represents standard length and TL total length

	Treatment	Lifestage	Fish	Weight (g)	SL (cm)	TL (cm)
Pyrolytic PAH	Control	juveniles	n=21	0.19±0.06	2.20±0.19	2.68±0.35
		adults	n=16	0.68±0.21	3.13±0.25	3.88±0.35
	3X	juveniles	n=22	0.17±0.05	2.10±0.26	2.59±0.26
		adults	n=15	0.48±0.13	2.81±0.24	3.50±0.22
Petrogenic PAH	Control	juveniles	n=15	0.09±0.03	1.82±0.28	2.26±0.34
		adults	n=19	0.30±0.06	2.62±0.19	3.29±0.23
	3x	juveniles	n=12	0.07±0.03	1.55±0.26	1.97±0.29
		adults	n=11	0.12±0.04	1.79±0.33	2.27±0.32

196

197 *2.4.3. Calculations*

198 *2.4.3.1. Critical swimming speed*

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

200

$$201 \quad U_{crit} = U_t + t_1 \cdot t^{-1} \cdot U_1 \quad \text{Equation (1)}$$

202

203 Where U_t (in $BL \cdot s^{-1}$) is the highest velocity maintained for an entire step, t_1 (in min) is the
 204 time spent until the exhaustion of fish at the last step, t (in min) is the swimming period for
 205 each step (i.e. 20 min in the present study) and U_1 is the increment velocity (0.75 or 1.5
 206 $BL \cdot s^{-1}$).

207

208 *2.4.3.2. Oxygen consumption (MO_2)*

209 Oxygen consumption MO_2 is expressed in $mg \ O_2 \cdot g^{-1} \cdot h^{-1}$ and calculated using the following

210 formula:

211

$$212 \text{ MO}_{2\text{meas}} = \Delta[\text{O}_2] \cdot V \cdot \Delta t^{-1} \cdot M_{\text{meas}}^{-1} \quad \text{Equation (2)}$$

213

214 where $\Delta[\text{O}_2]$ (in $\text{mg O}_2 \cdot \text{L}^{-1}$) is the variation in oxygen concentration during the measurement

215 period Δt (in h), V (in L) is the volume of the respirometer minus the volume of the fish and

216 M_{meas} (in g) is the fish mass measured.

217 An allometric relationship exists between oxygen consumption and body mass, which permits

218 correction of $\text{MO}_{2\text{meas}}$ using the following formula:

219

$$220 \text{ MO}_{2\text{cor}} = \text{MO}_{2\text{meas}} \cdot (M_{\text{meas}} \cdot M_{\text{cor}}^{-1})^{1-b} \quad \text{Equation (3)}$$

221

222 where $\text{MO}_{2\text{cor}}$ (in $\text{mg O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) is the oxygen consumption related to a standard fish of 1 g

223 (M_{cor}), $\text{MO}_{2\text{meas}}$ (in $\text{mg O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$) is the oxygen consumption estimated for experimental fish

224 whose mass was M_{meas} (in g) and b is the allometric scaling exponent describing the

225 relationship between oxygen consumption and body mass. A previous study of Lucas et al.

226 (2014a) estimated b in zebrafish *D. rerio* from the relationship between AMR and body mass,

227 i.e. b_{AMR} , and from the relationship between SMR and body mass, i.e. b_{SMR} . We used $b_{\text{AMR}} =$

228 0.926 for the correction of AMR and $b_{\text{SMR}} = 0.965$ for the correction of SMR.

229

230 2.4.3.3. Standard metabolic rate, active metabolic rate and aerobic metabolic scope

231 Oxygen consumption is known to increase exponentially with swimming speed (Brett, 1964).

232 The following equation can be therefore fitted on data and allows the assessment of SMR:

233

$$234 \text{ MO}_2 = \text{SMR} \cdot \exp^{bU} \quad \text{Equation (4)}$$

235

236 where MO_2 is the oxygen consumption (in $mg\ O_2.g^{-1}.h^{-1}$), SMR is the intercept (i.e. the MO_2
237 when $U = 0\ BL.s^{-1}$), b is a constant and U is the swimming speed (in $BL.s^{-1}$).

238 AMR is evaluated as the maximum oxygen consumption measured during the swimming
239 challenge. AMS is the difference between AMR and SMR. U_{crit} , SMR, AMR and AMS were
240 assessed for each fish except for HO-PAH-exposed juveniles. No SMR data were indeed
241 recorded for these individuals, because of the small size of the fish (relative to the swim
242 tunnel volume) and the low quality of the oxygen signal. The HO exposure impaired the
243 growth of fish exposed to (Vignet et al. 2014) and consequently HO-PAH-exposed juveniles
244 were smaller than PY-PAH-exposed juveniles (Table 2).

245

246 2.5. Histological analysis

247 Analysis occurred after the swimming challenge, in older fish: 3-month-old juveniles for both
248 types of contamination, 9-month-old adults for PY-PAH and 7-month-old adults for HO-PAH.
249 A total of 20 juveniles per treatment (i.e. *control* and *X*) were analysed for PY- and HO-PAH
250 exposure. In adults, 30 fish per treatment were observed for PY-PAH, and 25 fish were
251 observed for HO-PAH. Fish were euthanized with a lethal dose of benzocaine ($100\ mg.l^{-1}$;
252 Sigma-Aldrich), fixed in 4 % formalin for 2 weeks, dehydrated and embedded in paraffin wax
253 (Daouk et al., 2011). For each fish, 4 serial $4\ \mu m$ -thick sections spaced from from about 250
254 μm , were routinely stained with haematoxylin-eosin-saffron (HES). The histological
255 appearance of cardiac and skeletal muscles of fish was then observed using a bright-field
256 microscope (Nikon Eclipse 90i, Champigny, France) equipped with 2X, 4X, 10X, 20X and
257 40X objectives and a SPOT digital camera (AZ microscope, London, UK). Lesions were
258 systematically recorded by a skilled veterinary pathologist (member of the the European
259 College of Veterinary Pathologists).

260

261 2.6. Statistical analysis

262 Statistical analysis was carried out using GraphPad Prism software. For AMR, SMR, AMS
263 and U_{crit} , normality and homoscedasticity were checked using Kolmogorov–Smirnov and
264 Bartlett tests, respectively, for each duration of exposure and for each type of contamination
265 (i.e. *PY* and *HO*). Student’s t-tests were used to compare individuals exposed to the *control*
266 treatment with individuals exposed to PAH. Chi-square tests were used to compare
267 histological anomalies (i.e. cardiac and skeletal muscle structures) between *control* fish and
268 those exposed to *PY*-PAH or *HO*-PAH. For all tests, the results were considered to be
269 significantly different when $p < 0.05$.

270

271 3. Results

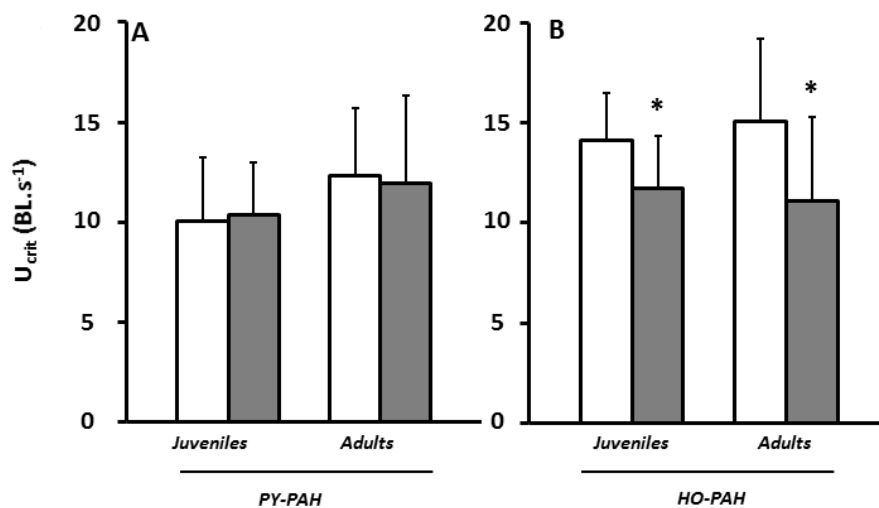
272

273 3.1. U_{crit}

274

275 Whatever the duration of exposure, no significant difference was found between fish exposed
276 to *control* or *PY*-PAH (Student’s t-test, degrees of freedom (dof) = 41 and $p = 0.34$ for
277 juveniles; dof = 26 and $p = 0.40$ for adults; Fig. 1A). On the contrary, U_{crit} decreased
278 significantly in both *HO*-PAH-contaminated juveniles and adults compared to *control*-
279 exposed individuals (Student’s t-test: dof = 24 and $p = 0.011$, dof = 28 and $p = 0.01$,
280 respectively; Fig. 1B).

281



282

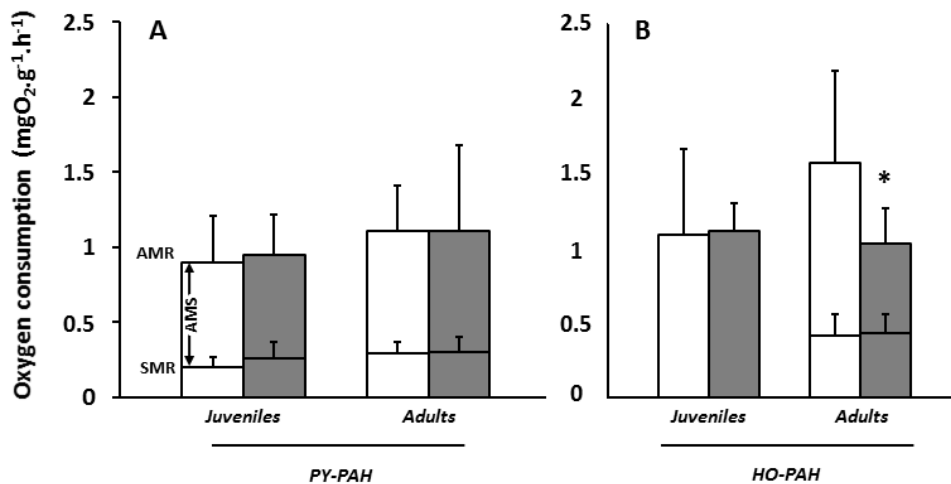
283 **Fig. 1** Critical swimming speed (U_{crit}) of zebrafish exposed to control (white bars) or to PAHs from
 284 pyrolytic (A) or petrogenic (B) origins (in grey). PAHs concentrations in food was 3X; the
 285 environmental reference concentrations X corresponding $X_{py}=17305\pm4798\mu\text{g}\cdot\text{g}^{-1}$ and
 286 $X_{pe}=8082.5\pm305\mu\text{g}\cdot\text{g}^{-1}$ of dry food (table 1). U_{crit} was evaluated in juvenile and adults. Results are
 287 expressed as mean values \pm standard deviation. For a given type of PAHs (PY or HO) and a given
 288 duration, * indicates a significant difference in U_{crit} between control and 3X treatment.
 289

290

291 3.2. Aerobic metabolic scope, standard and active metabolic rate

292

293 For PY-PAH contamination, no significant difference was found between contaminated fish
 294 and the control whatever the metabolic rate observed: SMR (Fig. 2A; Student's t-test: dof =
 295 32 and $p=0.40$, dof = 26 and $p=0.46$, for juveniles and adults, respectively), AMR (Fig. 2A;
 296 Student's t-test: dof = 34 and $p=0.29$, dof = 26 and $p=0.44$ for juveniles and adults,
 297 respectively) and AMS (Student's t-test: dof = 34 and $p=0.29$, dof = 26 and $p=0.44$ for
 298 juveniles and adults, respectively).



300

301 **Fig. 2.** Standard metabolic rate (SMR), active metabolic rate (AMR) and aerobic metabolic scope
 302 (AMS) of zebrafish *Danio rerio* exposed to control food (white bars) containing, pyrolytic (A) or
 303 petrogenic (B) PAHs (grey bars). PAH concentrations in food was X; the environmental reference
 304 concentrations corresponding to $X_{py}=17305\pm4798\mu\text{g.g}^{-1}$ and $X_{pe}=8082.5\pm305\mu\text{g.g}^{-1}$ of dry food
 305 (table 1). Aerobic metabolic rate was estimated at two life stages: juvenile and adults. Results are
 306 expressed as mean values \pm standard deviation. For a given type of PAHs (PY or HO) and a given
 307 duration, * indicates a significant difference in AMR between control and X treatment.
 308

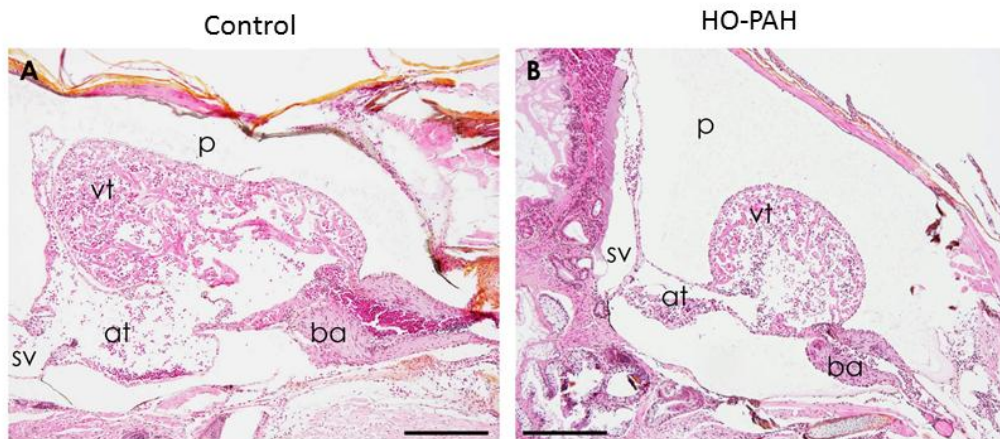
309 In *D. rerio* which ingested HO-PAH-contaminated food, the lack of SMR data for juveniles
 310 did not allow estimation of SMR and AMS for this stage. In adults, SMR did not differ
 311 significantly between control and contaminated fish (Fig. 2B; Student's t-test: dof = 27 and p
 312 = 0.35). On the contrary, AMR was significantly impaired, but only in adults (Student's t-test:
 313 dof = 20 and p = 0.43, dof = 27 and p = 0.006, for juveniles and adults, respectively; Fig. 2B),
 314 as was AMS (Student's t-test: dof = 27 and p = 0.003).

315

316 3.3. Histological analysis

317

318 During histopathological examination, no anomalies were observed in skeletal muscle for
319 either *PY*- or *HO*-PAH exposure. While no heart damage was observed for *PY*-PAH exposure,
320 4 out of 25 fish exposed to *HO*-PAH for 7 months displayed generalized oedema that severely
321 thickened the peritoneum all around the abdominal cavity. Significant heart impairments were
322 associated with this oedema: the pericardium appeared dilated with fluid and the heart was
323 very small and rounded with a thin ventricular wall (Fig. 3; chi-square test, dof = 1 and p =
324 0.002). These lesions are characteristic of cardiac insufficiency with secondary collection of
325 fluid in connective tissue and body cavities.



326

327 **Fig. 3** Heart of zebrafish exposed to control condition of food (A) or to PAHs from petrogenic origin
328 (B). Compared to a control fish, some fish exposed to PAHs displayed a large pericardial cavity with a
329 small rounded heart. at: atrium; ba: bulbus arteriosus; p: pericardium; sv: sinus venosus; vt: ventricle.
330 Hemalun-Eosin-Saffron. Bars = 200 μ m.

331

332

333 4. Discussion

334

335 Under these experimental conditions and at the two life stages tested, trophic exposure to
336 PAH differently impaired the fish depending on the type of PAH, i.e. PY or HO, and the
337 considered traits. AMR and U_{crit} were indeed influenced by HO-PAH, but SMR was not.

338 First, for a general comparison, swimming performance data were in the range of what other
339 authors have observed in *D. rerio*, since average U_{crit} ranged between 10 and 16.5 $BL.s^{-1}$ in
340 *control* individuals (e.g. $U_{crit} = 13 BL.s^{-1}$ in Plaut and Gordon, 1994; $U_{crit} = 15.5 BL.s^{-1}$ in
341 Plaut, 2001); $U_{crit} = 18 BL.s^{-1}$ in Palstra et al., 2010).

342 AMR values in *control* fish ranged between 0.9 and 1.6 $mg O_2.h^{-1}.kg^{-1}$, i.e. 30 to 60 lower
343 compared with other studies in the same species (Thomas and Janz, 2011, Lucas et al., 2014a,
344 2016). Such differences observed in AMR estimation could be explained by differences in the
345 general physiological status of the tested zebrafish (e.g. related to age, thermal acclimatization,
346 rearing conditions), as well as in the protocols used. It is also worth noting that Peake and
347 Farrell (2006) underlined the importance of behavioural regulations in assessing fish
348 performance through swimming challenges. They suggested that behaviour, rather than
349 physiological exhaustion, participates in the fatigue observed in fish tested in a swim flume,
350 thereby increasing the variability of the measured parameters (i.e. AMR and U_{crit}).

351

352 Focussing on contamination, HO-PAH exposure induced a significant decrease of 15 and
353 26.5% of U_{crit} in zebrafish exposed for 2 and 6 months, respectively. A similar pattern was
354 observed in the literature regarding pollutant effects (Cheng and Farrell, 2007; Hicken et al.,
355 2011; Howard, 1975; Marit and Weber, 2011, 2012; Thomas and Janz, 2011). In HO-PAH
356 adults, this was associated with a significant reduction of AMR, cardiac lesions (i.e. a smaller

357 and more rounded heart with a thin ventricular wall), generalized oedema and collection of
358 fluid in body cavities indicating a functional cardiac insufficiency. These lesions may induce a
359 reduction of cardiac performance and capacity to transport oxygen. This may limit aerobic
360 metabolism, especially during high levels of activity, such as occurs during swimming (Farrell
361 1997, 2007; Fry, 1947, 1971; Claireaux et al., 2005; Priede, 1985).

362 Even if U_{crit} was reduced in HO-PAH juveniles, no impairment of AMR or cardiac function
363 was observed. It is worth noting that U_{crit} depends on both aerobic and anaerobic metabolism
364 (Webb, 1998), which could explain the results observed. However, the anaerobic part of the
365 metabolism required to attain U_{crit} was not assessed in the present study, nor was oxygen debt
366 after the swimming test (e.g. Vagner et al., 2008). Indeed, it would have been interesting to
367 assess whether reduction of U_{crit} was associated to a different sollicitation of anaerobic
368 metabolism in juveniles and adults'

369

370 Contrary to the HO-PAH mixture, exposure to PY-PAH did not impair fish metabolic and
371 swimming performance or cardiac anatomy. The lack of effect could be explained by the fact
372 that organisms that suffer long-term chronic environmental stress can present physiological
373 adaptations to maintain their homeostasis. Chronic exposure to PY-PAH may have induced
374 such adaptations in *D. rerio*.

375 Whatever the type of exposure, no effect was observed on SMR. The range of observed
376 values are in agreement with previous studies carried out for the same species and with a
377 similar experimental approach ($SMR = 0.19 \text{ mg O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ in Barrionuevo and Burggren,
378 1999; mean \pm s.d., $SMR = 0.31 \pm 0.11$ and $0.35 \pm 0.16 \text{ mg O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ in juveniles and adults,
379 respectively, in Lucas et al., 2014a, 2016).

380 Even though the concentration of PAH and their metabolite compounds was not assessed in
381 juveniles and adults in this study, they were estimated in 15 dpf larvae exposed to HO- or PY-

382 PAH, and ranged between 42 ng.g⁻¹ and 136 ng.g⁻¹, respectively (Vignet et al., 2014).

383

384 It is likely that PAH metabolite concentration increased proportionally with the duration of
385 exposure. However, the present results suggest that the level of contamination tested was not
386 sufficiently extreme to induce significant variation in SMR in our experimental conditions.,
387 PAH exposure did not induce supplementary costs of maintenance due to detoxification
388 processes in zebrafish (Lannig et al., 2006; Lucas et al., 2016; Sokolova and Lannig, 2008;
389 Wilson et al., 1994), which is contrary to one of the initial hypotheses.

390 The different impairment of the types of PAH mixture the fish were exposed to could be
391 explained by their different chemical properties depending on the composition of individual
392 PAH (Vignet et al., 2014). It is worth noting that toxicity depends on individual intrinsic
393 properties of each component and their interactions with enzymatic systems in organisms.
394 Mainly characterized by four- to six-ring PAH and 5% of methylated compounds, PY-PAH
395 could induce less toxic effects than HO-PAH despite its higher concentration (Table 1).
396 Indeed, HO-PAH mixtures were characterized by three- and four-ring PAH, and 47% of
397 methylated compounds, which are considered to be more toxic for organisms (Di Giulio and
398 Hinton, 2008; Hall and Oris, 1991; Hylland, 2006; Tuvikene, 1995). In addition, adults that
399 were exposed to PAH for 6 months were therefore expected to contain more PAH metabolites
400 than juveniles, which coped with PAH exposure for only 2 months. This may contribute to the
401 fact that exposure to HO-PAH induced a significant reduction of AMR in adults and not in
402 juveniles. However, this pattern was not observed in juveniles and adults exposed to PY-PAH
403 (Fig. 1B) as well as in the similar study of Lucas et al. (2016).

404

405

406 **5. Conclusion**

407

408 Our results suggest that, contrary to HO-PAH, PY-PAH have no direct effect on aerobic
409 metabolism and swimming performance. Lucas et al. (2014b) have observed impairment of
410 metabolic performance and cardiac activity on the progeny of PY-PAH-contaminated *D. rerio*
411 parents. The parental effect of PAH exposure appears to be an important subject to study.

412 This study on swimming performance and associated energetic costs will be completed by
413 investigations regarding escape performance. This type of swimming depends on anaerobic
414 metabolism, as well as on sensorial and locomotion performance. Moreover, interfering with
415 some physiological mechanisms, PAH could be narcotic, inducing lethargic behaviour and
416 impairing the swimming performance of fish (Wassenberg and Di Giulio, 2004). All these
417 studies will permit a global view of the impact of this type of trophic contamination across
418 zebrafish generations.

419

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427

428

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