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

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ABSTRACT

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

1. Introduction

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

This study aimed at investigating responses of fish exposed to environmentally relevant concentrations of PAH through the assessment of AMS and U_{crit} as indicators of the physiological state of the organism (Fry, 1947). The increase of anthropogenic activities in coastal areas induces discharges of PAH in aquatic ecosystems. Due to their high liposolubility, PAH are typically adsorbed by organic matter or marine sediments, bioaccumulated by organisms at the lowest trophic levels (e.g. invertebrates; Bustamante et al., 2012; O'Connor and Lauenstein, 2006) and transferred through trophic chains (Hylland, 2006; Vignet et al., 2014). Past studies on fish have demonstrated that hydrocarbon exposure causes a decrease of U_{crit}. For instance, *Danio rerio* showed a 15% and 22% reduction of U_{crit} while exposed to crude oil (Hicken et al.,

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

PAHs	Ring	Molecular weight (g mol^{-1})	Concentration in dry food (ng g^{-1})			
			pyrolytic PAH		petrogenic PAH	
			Control	X	Control	X
Naphtalene	2	128.2	6 ± 6	157 ± 74	4 ± 1	405 ± 73
Acenaphthylene	2	152.2	1 ± 0	114 ± 23	1 ± 0	13 ± 13
Acenaphtene	2	154.2	2 ± 1	89 ± 24	27 ± 26	190 ± 17
Fluorene	2	166.2	2 ± 1	137 ± 28	2 ± 1	312 ± 25
Dibenzothiophenes	2	184.26	2 ± 3	102 ± 26	0 ± 0	546 ± 54
Phenanthrene	3	178.2	8 ± 4	895 ± 213	6 ± 3	1279 ± 51
Antracene	3	178.2	1 ± 0	482 ± 165	0 ± 0	220 ± 10
Fluoranthenes	3	202.3	3 ± 3	1782 ± 353	2 ± 1	145 ± 18
Pyrenes	4	202.3	3 ± 3	1496 ± 311	1 ± 0	709 ± 64
Benzo[*a*]anthracene	4	228.3	1 ± 0	1671 ± 763	0	543 ± 29
Chrysene + Triphenylene	4	228.3	1 ± 0	2144 ± 1032	ND	1073 ± 79
Benzonaphthothiophene	4	252.3	5 ± 3	472 ± 230	0	573 ± 36
Benzo[*b*,*k*,*j*]fluoranthenes	4	252.3	2 ± 1	2740 ± 674	2,4	363 ± 18
Benzo[*e*]pyrene	5	252.3	1 ± 0	1084 ± 286	ND	536 ± 26
Benzo[*a*]pyrene	5	252.3	1 ± 0	1168 ± 346	0 ± 0	342 ± 10
Perylene	5	252.3	1 ± 0	390 ± 83	0 ± 0	172 ± 14
Indeno[1,2,3-cd]pyrene	5	276.3	0 ± 0	1188 ± 265	ND	ND
Dibenzo[*a*]anthracene + Dibenzo[*a*]chrysene	5	276.3	2 ± 2	301 ± 106	0,3	113 ± 6
Benzo[*g*,*h*, *i*]perylene	6	276.3	0 ± 0	893 ± 191	0 ± 0	481 ± 12
Sum PAHs			**34 ± 6**	**17,305 ± 4798**	**39 ± 30**	**8082 ± 305**

2011) and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin; Marit and Weber, 2012), respectively. Moreover, PAH could induce damage of tissues and/or organs involved in oxygen transport (e.g. fish gills, Fasulo et al., 2012; mussel gills, Cappello et al., 2013). This could induce a decrease of AMR as observed by Davoodi and Claireaux (2007) after acute PAH exposure of *Solea solea*. In addition, the implementation of detoxification processes which are energy costly have already been demonstrated. These processes are likely to increase costs of maintenance and, consequently, SMR. The consequent potential reduction of AMS would reflect a decrease in the capacity of fish to sustain energy-demanding activities.

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

2. Materials and methods

2.1. General production of zebrafish

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

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

2.2. Food contamination protocol

Artificial dry food (INICIO Plus, BioMar France) was spiked with mixtures of PAH as described in detail by Vignet et al. (2014). Briefly, the pyrolytic fraction (*PY*) was extracted from natural sediment sampled at the site of Oissel (Seine Bay, France), while the petrogenic fraction (*HO*) was extracted from heavy Erika fuel oil. For both types of *PY* and *HO* mixtures, quantification of the 16 individual PAH of the US EPA list was made, according to a protocol described in Budzinski et al. (1997) and Cachot et al. (2007). The food was then spiked with the extracted *PY* or *HO* fractions according to a protocol adapted from Vicquelin et al. (2011). The target concentration was 15,000 ng g^{-1} of food and based on the sum of the 16 PAH from the US EPA list in dry weight. This corresponds to three times the PAH concentration organisms may be exposed to in their natural environment (Cachot et al., 2006; Cailleaud et al., 2007; Payne et al., 2008). This high concentration was used to highlight the toxicological effects on the physiological performances investigated in this study. It is called *X* in the rest of the manuscript. Food size was adapted to the developmental stage of *D. rerio*. A *control* treatment was also considered, and consisted of feeding groups of fish with uncontaminated food, treated only by the solvent dichloromethane. This solvent was used to improve PAH incorporation into the food, and then evaporated. PAH compositions and concentrations in the diet are described in detail in Table 1.