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Mathilde Pigneret, Damien Roussel, Frédéric Hervant. Anaerobic end-products and mitochondrial parameters as physiological biomarkers to assess the impact of urban pollutants on a key bioturbator. *Environmental Science and Pollution Research*, 2018, 25 (27), pp.27225-27234. 10.1007/s11356-018-2756-x . hal-01953046

HAL Id: hal-01953046

<https://sde.hal.science/hal-01953046v1>

Submitted on 3 Oct 2024

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Anaerobic end-products and mitochondrial parameters as physiological biomarkers to assess the impact of urban pollutants on a key bioturbator

Mathilde Pigneret¹ · Damien Roussel¹ · Frédéric Hervant¹

Abstract

The impact of long-term exposure (6 months) to highly or slightly polluted sediments on the energy metabolism of an ecosystem engineer (the oligochaete *Limnodrilus hoffmeisteri*) was investigated in laboratory conditions. We evaluated some mitochondrial parameters (respiratory chain activity and ATP production rate) and the accumulation of anaerobic end-products (lactate, alanine, succinate, and propionate). The sediments were collected from stormwater infiltration basins and presented high levels of heavy metals and polycyclic aromatic hydrocarbons (PAHs). These compounds had been drained by the runoff water on impervious surfaces of urban areas during rainfall events. A decrease in the activity of the mitochondrial electron transport chain was observed in worms exposed to the most polluted sediment. Urban contaminants disrupted both aerobic metabolism and mitochondrial functioning, forcing organisms to shift from aerobic to anaerobic metabolism (which is characteristic of a situation of functional hypoxia). Although *L. hoffmeisteri* is very tolerant to urban pollutants, long-term exposure to high concentrations can cause disruption in mitochondrial activity and therefore energy production. Finally, this study demonstrated that anaerobic end-products could be used as biomarkers to evaluate the impact of a mixture of urban pollutants on invertebrates.

Keywords Urban pollution · Anaerobic metabolism · Mitochondria · Biomarkers · Metabolic shift · Tubificid worms

Introduction

Numerous studies (mainly on fish and invertebrates) have demonstrated the influence of toxic stress on metabolic pathways (De Coen et al. 2001, and references therein; Giacomini et al. 2014, and references therein). In oxygen-dependent animals, the oxidative metabolism of carbohydrates is the main energy-generating pathways (Hochachka and Somero 2002), but pollutants have long been recognized as powerful disrupters of carbohydrate metabolism and, more broadly, of cell respiration (Gagnon and Holdway 1999; Grajeda y Ortega et

al. 2011; Meyer et al. 2013; Giacomini et al. 2014). For example, alterations of glycolysis and/or of citric acid cycle functioning have been observed in several fishes living in polluted environments (review in Gagnon and Holdway 1999). Mitochondria are essential organelles, playing critical roles in numerous key processes (including ATP generation), but recent studies indicate that they are also targeted by environmental toxicants (review in Meyer et al. 2013). Thus, some pollutants, among which polycyclic aromatic hydrocarbons (PAHs) and heavy metals (mainly lead and cadmium), have been shown to preferentially accumulate in mitochondria because of the high lipid content of mitochondrial membranes (PAHs) or through calcium transporters (heavy metals) (Schaller et al. 2011; Gauthier et al. 2014). In the same way, metals (Cd, Cu, Zn, and Fe) affect the activity of iron-dependent mitochondrial enzymes (mainly succinate dehydrogenase and NADH-dehydrogenase) (Giacomini et al. 2014). The reduction or induction of these catalytic activities alters both energy metabolism and cell respiration, thereby significantly decreasing ATP production rate (review in Grajeda y Ortega et al. 2011) with potential effects on

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survival, growth, activity, and/or reproduction. In addition, Lauer et al. (2012) reported a decrease in both the activity of key enzymes involved in both the energy metabolism and in the mitochondrial membrane potential in the estuarine crab *Neohelice granulata* exposed to a sublethal concentration of copper, which could lead to a reduced mitochondrial ATP production. Unfortunately, little attention has been paid to the impact of pollutants on ATP generation.

In urban areas, the high impervious surface coverage disturbs the infiltration of runoff water during rainfall events (Ku et al. 1992; Niemczynowicz 1999). Consequently, stormwater management structures—mainly infiltration basins—were built to collect runoff water and recharge groundwaters (Marsalek and Marsalek 1997; Marsalek and Chocat 2002). These structures are of great ecological importance because they permit to restore the water cycle in urban zones. But runoff water also drains numerous pollutants (mainly hydrocarbons and heavy metals). These contaminants are accumulated in the sediment compartment of infiltration/stormwater basins, altering its quality and impacting organisms living in this harsh biotope (Pignernet et al. 2016). In particular, changes in physiology and burrowing activities impair the ability of ecosystem engineers—mainly tubificid/bioturbator worms—to influence sediment biogeochemistry, and therefore affect the functioning of ecosystems receiving stormwater from urban areas (Mermillod-Blondin et al. 2013; Pignernet et al. 2016). Among these organisms, the oligochaete *Limnodrilus hoffmeisteri* is usually exposed to urban pollutants in infiltration basin sediments and is considered as a key bioturbator (Datry et al. 2003; Pignernet et al. 2016, and references therein). This aquatic worm is thought to have an excellent potential for the evaluation of pollutant toxicity (Grajeda y Ortega et al. 2011, and references therein). According to Datry et al. (2003b) and Pignernet et al. (2016) who did not detect mortality in tubificid worms incubated in stormwater sediments, we did not expect that urban pollution would influence the survival of tubificid worms, but rather that it would significantly impact their physiology.

In the present study, we studied the effect of a mixture of pollutants (mainly heavy metals and PAHs) on the energy metabolism of *L. hoffmeisteri*. We predicted that a long-term exposure to urban toxicants would disrupt both aerobic metabolism and mitochondrial functioning, resulting in a partial or a total transition to anaerobic metabolism. This hypothesis was tested by assessing the possible changes induced by polluted sediments on the level of the main anaerobic end-products (lactate, alanine, succinate, and propionate (Schöttler and Bennet 1991; Datry et al. 2003)), mitochondrial respiration, ATP production rate, and mitochondrial oxidative phosphorylation efficiency in *L. hoffmeisteri*. The experiments were conducted during 6 months under laboratory conditions, in microcosms. Tubificid worms were exposed to three polluted sediments (from three infiltration basins) and

one slightly polluted sediment (from a stream) considered as a control.

Material and methods

Model organism

The worm *L. hoffmeisteri* (Annelidae, Tubificidae) (Claparède, 1862) was selected because it is tolerant to pollutants (Lotufo and Fleeger 1996; Millward et al. 2001; Pignernet et al. 2016) and is recognized as a key bioturbator in freshwater sediments. This species is a common sediment feeder that creates biogenic structures in sediments and egests fecal pellets at the water-sediment interface (McCall and Tevesz 1982; Mermillod-Blondin et al. 2001; Ciutat et al. 2006; Shang et al. 2014). This behavior influences ecosystem functioning (Mermillod-Blondin and Rosenberg 2006; Mermillod-Blondin and Lemoine 2010). Because of these characteristics, this tubificid worm was recognized as a pertinent model organism (Pignernet et al. 2016). Specimens of *L. hoffmeisteri* (mean individual dry mass 350–400 µg) used in our experiments were bought from a professional breeder (GREBIL, Arry, France). Before of the experimentation, they were acclimated to sedimentary habitat in 100-L plastic tanks (“acclimation tanks”) containing fine slightly polluted sediment (25 L) from the Ain river (Ain, France) and normoxic water (50 L, bubbled with air near the water surface) during 15 days (see Pignernet et al. 2016 for details concerning the acclimation sediment).

Sediment characteristics

We selected four sediments (three stormwater sediments and one stream sediment) to test the influence of contrasted pollutant concentrations on the survival and physiology of tubificid worms. The three polluted sediments were collected in three infiltration basins (Django-Reinhardt, IUT, and Minerve basins) located in industrialized and urbanized areas of the “Metropole de Lyon” conurbation (France; see Marmonier et al. 2013 for details concerning these basins). The control sediment (called BAL) was collected from a blind channel of the Rhône River (Balan, France). All four sediments were sieved (maximum diameter 1 mm) and homogenized in the laboratory, and then, their particle sizes distributions were determined using a laser diffraction granulometer (Mastersizer 2000, Malvern Instrument, UK) according to Gette-Bouvarot et al. (2014). Total organic carbon and nitrogen concentrations were quantified by high-temperature combustion of pre-acidified dry samples and subsequent measurement of CO₂ and N₂ by thermal conductivity using an element analyzer (FlashEA, Thermo Electron Corporation). These analyses showed that granulometry, total inorganic carbon, and nitrogen were

similar between all the sediments. Metals (Cu, Zn, Cd, and Pb) in sediments were extracted using a microwave-assisted digestion with HNO₃ and HCl and analyzed by ICP-AES (Thermo Electron Maxim) (Pigneret et al. 2016). PAHs in sediments were analyzed using high-performance liquid chromatography (HPLC) with fluorescence detector (AGILENT 1100) following cold extraction with CCl₄ (Pigneret et al. 2016). These analyses showed that the concentration of PAHs (mainly pyrene) is about 4.5 times higher in IUT basin (called IUT) than in both Django-Reinhardt (called DJ) and Minerve (called MIN) basins due to different urban area catchments

(Marmonier et al. 2013). The control sediment (BAL) was characterized by a low concentration of PAHs and heavy metals (Table 1). In comparison, higher concentrations of total hydrocarbons (BAL concentrations × 23), heavy metals (× 27), and PAHs (× 12) characterized the three stormwater sediments (see Table 1).

Experimental design

The experimental design was realized as described in Pigneret et al. (2016), with the four sediments (BAL, MIN, IUT, and

Table 1 Physical and chemical characteristics of the four sediments (mean ± sd, *n* = 1–3)

	Balan	Minerve	IUT	Django
Grain size distribution (%)				
Sand	75.2 ± 2.5	77.5 ± 2.7	72.3 ± 2.2	76.5 ± 4.5
Silt	24.2 ± 2.4	22.0 ± 2.6	27.1 ± 2.2	22.9 ± 4.3
Clay	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.2
Organic matter composition (% sed. DM)				
Total organic carbon	7.89 ± 0.57	11.98 ± 0.15	8.12 ± 1.01	12.41 ± 0.09
Total nitrogen	0.64 ± 0.06	0.72 ± 0.01	0.50 ± 0.04	0.76 ± 0.01
Pollutant contents				
Total hydrocarbons C10–C40 (mg kg ⁻¹ sed DM)	178	3,348	1,067	7,743
Heavy metals (mg kg ⁻¹ sed. DM)				
Cadmium	0.3			
7.82	0.8	2.9	2.3	
Copper	7.82	300	179.6	314.5
Lead	15	122	210.6	198.6
Zinc	50.6	1,397	764.6	2,528
Σ heavy metals	73.72 ± 31.4	1,819.8 ± 824.4	1,157.7 ± 482	3,043.4 ± 1,447.5
PAHs (µg kg ⁻¹ sed. DM)				
Acenaphthylene	< 10	< 10	< 10	< 10
Fluoranthene	102	362	2,314	551
Benzo (b) fluoranthene	84	368	1,214	436
Benzo (k) fluoranthene	27	119	540	161
Benzo (a) pyrene	49	178	1,145	263
Benzo (ghi) perylene	57	575	968	562
Indeno (1,2,3-cd) pyrene	47	319	981	385
Anthracene	13	21	189	23
Acenaphthene	< 10	< 10	215	< 10
Chrysene	72	278	1,692	368
Dibenzo (a,h) anthracene	< 10	< 10	400	< 10
Fluorene	< 10	< 10	189	< 10
Naphtalene	< 10	< 10	< 10	< 10
Pyrene	83	501	2,186	633
Phenanthrene	48	232	1,789	325
2-Methyl fluoranthene	< 10	< 10	< 10	< 10
Benzo (a) anthracene	39	113	1,101	192
Σ PAHs	621 ± 26.6	3,066 ± 168.9	14,923 ± 720.9	3,899 ± 187.1

PAHs polycyclic aromatic hydrocarbons, DM dry mass

DJ) and four durations of exposure to pollutants: 1 month (M1), 3 months (M3), 6 months (M6), and the initial control (M0). For metabolic and mitochondrial analyses, worms were exposed to the polluted (MIN, IUT, or DJ) or slightly polluted (BAL) sediments in 80-L glass tanks (“incubation tanks”), with the density of 26,000 worms/m². Three incubation tanks were set up for each sediment. Each incubation tank was filled with a 5-cm-thick layer of sediment overlaid by 5 cm of synthetic water (96 mg L⁻¹ NaHCO₃, 39.4 mg L⁻¹ CaSO₄ + 2H₂O, 60 mg L⁻¹ MgSO₄ + 7H₂O, 4 mg L⁻¹ KCl, pH = 7.5; US EPA 1991) bubbled with air near the water surface. One incubation tank of each sediment was dismantled at each time point (M1, M3, and M6). To measure physiological indicators, tubificid worms were collected in the incubation tanks after 1, 3, or 6 months of exposition to polluted or low polluted sediments. Control (M0) organisms were sampled at the start of the experiment, directly in the acclimation tanks, in order to determine their initial physiological state. Then, the worms were placed in filtered water from their original tanks during 24 h to avoid measuring the metabolic contents in their digestive bolus (sediment) in addition to the metabolite content of the whole animal (Plénet et al. 2000; Renault et al. 2002; Pignieret et al. 2016). After this period, the worms were separated into two groups. Those of the first group were freeze-dried (Christ lyophilizator, ALPHA 1-4LD) and weighed (dry mass) prior to anaerobic end-products assays. Those of the second groups were directly used for the measurement of mitochondrial parameters.

Anaerobic end-product assays

To measure alanine, succinate, propionate, and lactate contents, pools of six freeze-dried worms were homogenized in 1 mL of ice-cold perchloric acid (0.33 N) using an ultraturrax (Janke & Kunkel, IKA-WERK), as described by Hervant et al. (1995, 1997). All assays were performed using a spectrophotometer (Thermo Electron Corporation, AQUAMATE) at 25 °C, using standard enzymatic methods as described by Bergmeyer (1985) and Hervant et al. (1995, 1997). Enzymes, coenzymes, and substrates used for enzymatic assays were purchased from Sigma-Aldrich (St. Louis, USA).

Mitochondrial parameters

Mitochondrial isolation

We collected (from the incubation tanks) 4 replicates of 500 pooled worms after 6 months of exposure to sediments (M6), for each sediment (BAL, MIN, IUT, and DJ). Mitochondria were isolated at 4 °C, in buffer containing 600 mM mannitol, 20 mM Tris-maleate, 1 mM EDTA, 2 mM EGTA, 2% w/v bovine serum albumin, pH 7.3, following standard differential centrifugation protocol (as described in Salin et al. 2012;

Colinet et al. 2017). Protein concentration was measured using the Biuret method with bovine serum albumin as a standard, at 540 nm.

Mitochondrial respiration and ATP production rate

Mitochondrial oxygen consumption was measured in respiratory buffer (120 mM KCl, 5 mM KH₂PO₄, 1 mM EGTA, 2 mM MgCl₂, 0.3% bovine serum albumin (*w/v*), and 3 mM HEPES, pH 7.4) using a Clark electrode (Rank Brother Ltd., Cambridge, UK) placed in a closed and stirred glass chamber (volume 500 µL) and expressed per mg of proteins at 15 °C. Pyruvate-malate-succinate (5/2.5/5 mmol L⁻¹) were used as respiratory substrates. The active state of respiration (mitochondrial state 3_{ADP}) and the maximum (uncoupled) respiration rate (state 3_{FCCP}) were determined after the addition of ADP (500 µmol L⁻¹) and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (2 µmol L⁻¹ FCCP), respectively (as described in Roussel et al. 2015).

Mitochondrial oxidative phosphorylation efficiency was determined by measuring ATP synthesis (*J*_{ATP}) concurrently to oxygen consumption in a respirometry buffer supplemented with 20 mM glucose, 1.5 U/mL hexokinase, and 500 µM ADP (as described in Salin et al. 2012; Colinet et al. 2017). Mitochondrial ATP synthesis was determined by assaying the linear glucose-6-phosphate accumulation in mitochondrial suspension over a sampling time interval of 6 min (as described in Salin et al. 2012; Colinet et al. 2017). The same procedure was performed with the addition of 1 µg mL⁻¹ oligomycin to ensure that the ATP production measured was specific to mitochondrial ATP synthase activity.

Statistical analysis

Results of the end-product concentrations are presented as means ± SE, and results of mitochondrial parameters are presented as boxplots (no outlier was detected). Anaerobic end-product data were log-transformed to homogenize variances when homoscedasticity and normality were not observed. To test the effect of sediment treatment (the type of mixture of pollutants is different between the sediments) and duration of exposure to pollutants on the anaerobic end-product concentrations, two-way analyses of variance (two-way ANOVA) were performed to test the effect of each explanatory variables (sediment treatment, duration of exposure) and the interaction between them (sediment treatment × duration of exposure). If significance was detected, Tukey’s HSD tests were performed to determine which sediment treatments and duration differed between each anaerobic end-product concentrations. In view of the small number of replicates (*n* = 4) during the mitochondrial experiments, Kruskal-Wallis (K-W) tests were performed to test the effect of the sediment treatments on mitochondrial

parameters. If significance was detected, Wilcoxon tests were performed for pairwise comparisons to determine which sediment treatments differed after 6 months of exposure. All statistical analyses were performed with the software R (RTM Development Core Team). Significance for all statistical tests was accepted at $\alpha < 0.05$.

Results

There was a negligible mortality (<5%) of worms during the 6-month period of experiment in the pollutant-exposed groups (MIN, IUT, and DJ) and in the control group (BAL). Therefore, all effects reported in the present study were considered to be sublethal.

Mitochondrial parameters

Oxygen consumption

Figures 1 and 2 present the different respiratory parameters of mitochondria isolated from each batch of 500 worms and exposed to the four sediments (BAL, MIN, IUT, and DJ). The phosphorylating respiration (state 3_{ADP}) (Fig. 1a) was significantly influenced by the sediment treatment (K-W test, $p = 3.96 \times 10^{-2}$). The value measured in worms exposed to the DJ sediment was significantly lower (–55%) than that of worms exposed to the BAL sediment (Wilcoxon test, BAL-DJ $p = 2.85 \times 10^{-2}$).

The rate of FCCP-induced maximal oxygen consumption (Fig. 2a) in presence of FCCP (state 3_{FCCP}) was influenced by

the sediment treatment (K-W test, $p = 2.57 \times 10^{-2}$). The value measured in worms exposed to DJ sediments was significantly lower than that of worms exposed to the BAL sediment (–68%) (Wilcoxon test, BAL-DJ $p = 4.20 \times 10^{-2}$).

ATP synthesis rate

The ATP synthesis rate (J_{ATP}) was not influenced by the sediment treatment (K-W test, $p = 5.08 \times 10^{-2}$) (Fig. 1b), but we can notice a trend to decrease in the ATP production rate as the p value (5.08×10^{-2}) is close to 0.05. We also noticed a trend to decrease in worms exposed to the DJ sediment (–54%) compared to worms exposed to the MIN sediment despite the statistical non-significance (Wilcoxon test, $p = 5.71 \times 10^{-2}$). The sediment treatment did not influence the ATP/O ratio (KW test, $p = 0.54$) (Fig. 2b).

Anaerobic end-products

The succinate body level of worms was also significantly influenced by both the duration of exposure and sediment treatments (Fig. 3a; two-way ANOVA, “duration” effect $p = 1.23 \times 10^{-7}$; “sediment treatment” effect, $p = 8.47 \times 10^{-3}$; “duration \times sediment” interaction, $p = 3.83 \times 10^{-2}$). The succinate concentration significantly increased after a 6-month exposure to polluted sediments (MIN, IUT, and DJ) in *L. hoffmeisteri*.

The propionate body level of worms was significantly influenced by both the duration of exposure to the sediment and sediment treatments (Fig. 3b; two-way ANOVA, duration effect $p = 2.13 \times 10^{-9}$; sediment treatment effect, $p = 1.41 \times$

Fig. 1 Mitochondrial respiration (state 3) (a) and ATP synthesis rate (J_{ATP}) (b) of *Limnodrilus hoffmeisteri* after a 6-month exposure to each sediment. BAL Balan sediment, MIN Minerve sediment, IUT IUT sediment, DJ Django sediment ($n = 4$; pool of 500 worms per n). Letters (a, b) indicate significant differences in pairwise comparisons between sediment treatments (Wilcoxon’s tests $p < 0.05$)

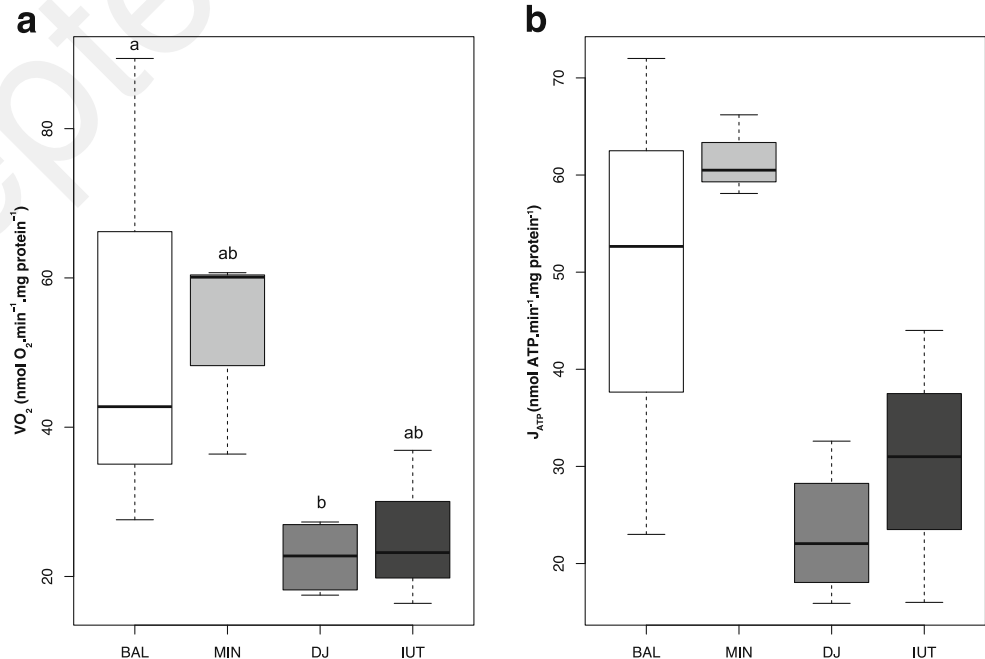
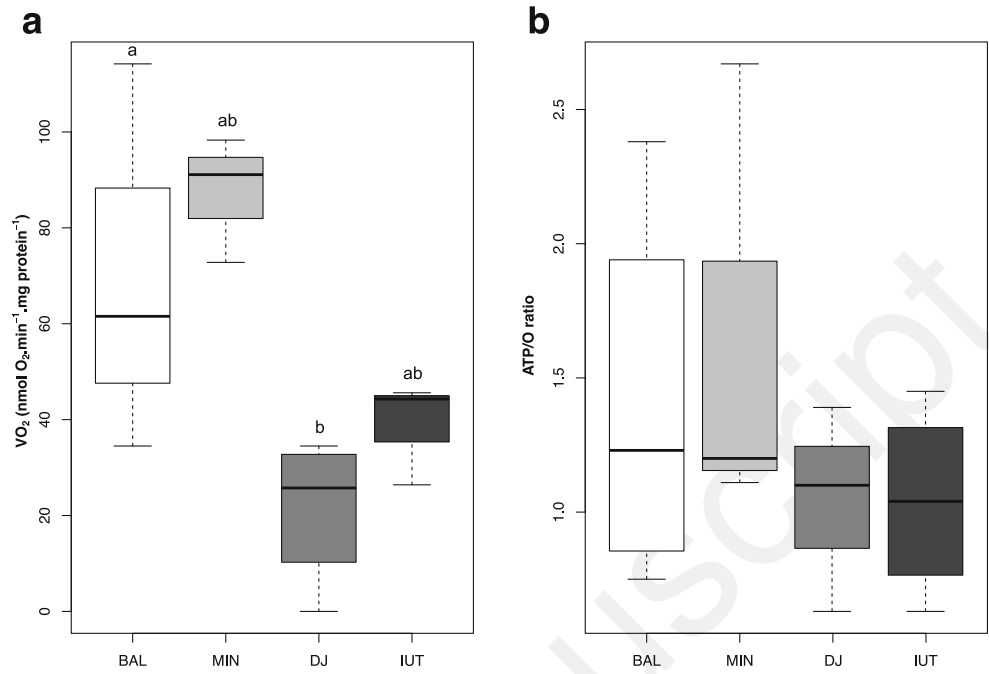


Fig. 2 The decoupling maximum activity of the electron transport chain (state 3_{FCCP}) (a) and ATP/O ratio (b) of *Limnodrilus hoffmeisteri* after a 6-month exposure to each sediment. BAL Balan sediment, MIN Minerve sediment, IUT IUT sediment, DJ Django sediment ($n = 4$; pool of 500 worms per n). Letters (a, b) indicate significant differences in pairwise comparisons between sediment treatments (Wilcoxon's tests $p < 0.05$)



10^{-3} ; duration \times sediment interaction, $p = 2.04 \times 10^{-2}$). The propionate concentration significantly increased after a 6-month exposure to polluted sediments (MIN, IUT, and DJ) in *L. hoffmeisteri*.

The lactate body level of worms was not influenced by the duration of exposure to the sediment and sediment treatments (Fig. 4a; two-way ANOVA, duration effect $p = 6.01 \times 10^{-1}$; sediment treatment effect, $p = 8.22 \times 10^{-1}$; duration \times sediment interaction, $p = 5.30 \times 10^{-1}$).

The alanine body level of worms was not influenced by the duration exposure to the sediment and sediment treatments (Fig. 4b; two-way ANOVA, duration effect $p = 1.42 \times 10^{-1}$; sediment treatment effect, $p = 8.06 \times 10^{-1}$; duration \times sediment interaction, $p = 6.62 \times 10^{-1}$).

Discussion

In this study, we measured the effect of urban pollutants on the energy metabolism of an ecosystem engineer, the bioturbator oligochaete *L. hoffmeisteri*. We hypothesized that a long-term exposure to a mixture of toxicants would disrupt both aerobic metabolism and mitochondrial functioning, resulting in a transition to anaerobic metabolism. This double hypothesis was tested by assessing the possible changes induced by pollutants on the level of the main anaerobic end-products, mitochondrial respiration, ATP production rate, and mitochondrial oxidative phosphorylation efficiency in *L. hoffmeisteri*. To this end, worms were exposed for 6 months, under laboratory

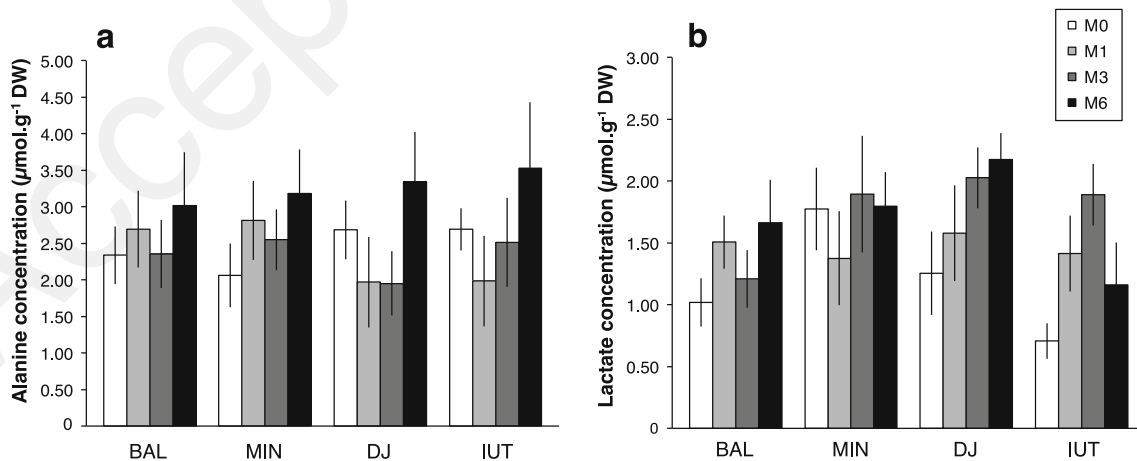


Fig. 3 Body concentrations of alanine (a) and lactate (b) in *Limnodrilus hoffmeisteri* after 0, 1, 3, and 6 months of exposure to each sediment. Data presented as means \pm s.e.m. ($n = 9$). BAL Balan sediment, MIN Minerve

sediment, IUT IUT sediment, DJ Django sediment. No significant difference in pairwise comparisons between sediment treatments for only the duration effect was observed (Tukey's HSD tests $p > 0.05$)

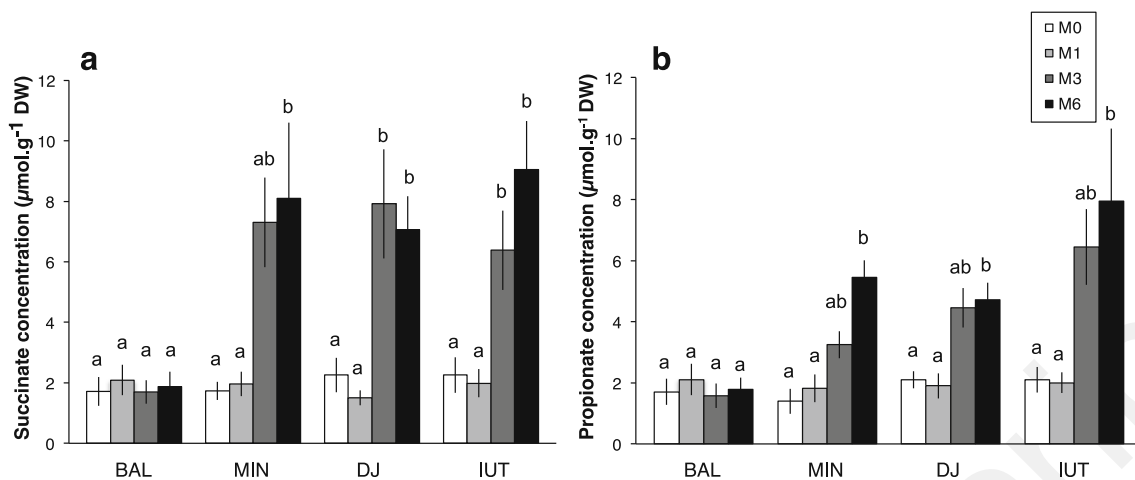


Fig. 4 Body concentrations of succinate (a) and propionate (b) in *Limnodrilus hoffmeisteri* after 0, 1, 3, and 6 months of exposure to each sediment. Data presented as means \pm s.e.m. ($n = 9$). BAL Balan sediment,

MIN Minerve sediment, IUT IUT sediment, DJ Django sediment. Letters (a, b) indicate significant differences in pairwise comparisons between sediment treatments for only the duration effect (Tukey's HSD tests $p < 0.05$)

conditions, to three polluted sediments and one slightly polluted sediment.

Impact of urban pollutants on mitochondrial functioning

A decrease in the whole mitochondrial oxidative phosphorylation activity was observed after 6 months in worms exposed to the most polluted sediments (stormwater basins IUT and DJ), resulting in a large decrease in oxygen consumption. The maximal activity of the electron transport chain, measured in the presence of FCCP (an uncoupling agent), was also negatively altered in worms exposed to the most polluted sediments (IUT and DJ). These results suggest that the mixture of contaminants found in those contaminated sediments altered the functioning of electron transport chain.

Other studies have also shown that metals and PAHs (mainly cadmium and benzo-(a)-pyrene) accumulate in and negatively affect mitochondrial functioning (Wang et al. 2004; Cannino et al. 2009; Kurochkin et al. 2011; Grajeda y Ortega et al. 2011; Meyer et al. 2013; Ji et al. 2016). Indeed, cadmium and PAHs can enter and accumulate in the mitochondrial compartment and/or in the mitochondria inner membrane (review in Meyer et al. 2013), potentially inducing a dysfunction in the respiratory chain. This property may partly explain the mitochondrial disturbance observed in the worms exposed to the most polluted sediments. Moreover, Li et al. (2003) stated that mitochondria are targeted by PAHs, as these compounds are lipophilic and are therefore attracted by lipid-rich membranes like those found in the mitochondria. One may suggest that lipophilic pollutants such as PAHs can interact with mitochondrial membranes and thus modify their functioning (Gauthier et al. 2014). Such structural modifications could create homeostasis disturbances related

to fluidity changes in the mitochondrial membranes (Guderly et al. 2008; Gauthier et al. 2014).

Some authors have demonstrated that a few urban pollutants affected the activity of the key enzymes involved in the aerobic metabolism (specific to glycolysis and Krebs cycle) (review in Meyer et al. 2013; Giacomini et al. 2014). Thus, Lauer et al. (2012) showed that an exposure to copper reduced hexokinase, pyruvate kinase, citrate synthase, and phosphofructokinase activities in the gills of *N. granulata*. Cadmium is also known to reduce the succinate dehydrogenase (a key enzyme of the Krebs cycle that links the mitochondrial respiratory chain to the citric acid cycle) activity in *L. hoffmeisteri* (Grajeda y Ortega et al. 2011). As a result, the inhibitory properties shown by urban contaminants on numerous key enzymes of aerobic metabolism could partly explain the depression of aerobic metabolism observed in the present study in the tubificid worm *L. hoffmeisteri*.

Transition to anaerobic metabolism induced by urban pollutants

Lauer et al. (2012) hypothesized that exposure of the euryhaline crab *N. granulata* to copper could lead to a physiological hypoxia and to a deficit in energy production. In the present study, a significant increase in the body concentrations of some anaerobic end-products (succinate and propionate) was observed after 3 months in worms exposed to polluted sediments, confirming the hypothesis of a malfunctioning of the aerobic metabolism, resulting in a transition to anaerobic metabolism. All these metabolic changes were induced by urban pollutants and were not observed in control worms. These findings demonstrate that exposure of the key bioturbator *L. hoffmeisteri* to urban contaminants leads to its increased reliance upon anaerobic energy production, thus maintaining for it a sufficient ATP production.

Surprisingly, the body concentrations of lactate and alanine remained stable in individuals of *L. hoffmeisteri* incubated in polluted sediments, while these anaerobic end-products were detected (in association with succinate and propionate) in the bioturbator oligochaete *Tubifex tubifex* after exposure to the polluted sediments from the same stormwater basins (Datry et al. 2003). Moreover, increased lactate dehydrogenase (LDH) activity or lactate accumulation following exposure to pollutants have been reported in a few aquatic invertebrates (Carvalho and Fernandes 2008; Lauer et al. 2012; Giacomini et al. 2014). The anaerobic pathway leading to lactate as an end-product was clearly not used by *L. hoffmeisteri*.

Glycolysis yields two molecules of ATP. As the first stage of carbohydrate metabolism, it also generates two molecules of pyruvate, which under aerobic conditions are oxidized to acetyl-CoA and enter the Krebs cycle. When oxygen is not available, pyruvate is generally reduced to lactate through LDH (and/or to alanine through alanine dehydrogenase), thus recycling NAD⁺ that can again be used by glycolysis (Nelson and Cox 2017). But some organisms optimize energy production by synthesizing other end-products leading to significant increases in ATP generation during physiological or environmental hypoxia. More precisely, lactate (or alanine) production results in the formation of 2 ATP/mol of glucose catabolized, but the production of succinate or propionate offers additional sites (1 or 2, respectively) for ATP production (Barret 1991; Schöttler and Bennet 1991). The present study clearly demonstrates that *L. hoffmeisteri* preferentially uses the most efficient (anaerobic) energy pathways to compensate for the mitochondrial dysfunction induced by urban pollutants, leading to a more than doubled ATP production.

Anaerobic energy production is always less efficient than its aerobic counterpart (Schöttler and Bennet 1991). Therefore, the shift from aerobic to anaerobic metabolism appeared to be clearly disadvantageous for worms submitted to polluted sediments. But this transition also limits the production of reactive oxygen species (ROS; free radicals) in cells, and therefore could represent an efficient response for worms to counteract the increased oxidative stress induced by exposure to urban pollutants (Pignoret et al. 2016). Thus, we could hypothesize that the reduced energy requirements for fighting the oxidative stress might partially compensate for the decreased energy production in worms incubated in polluted sediments.

Conclusion

The present study demonstrates that urban polluted sediments displayed a variety of negative effects on both mitochondrial functioning and energy metabolism in the key bioturbator *L. hoffmeisteri*. But this worm was able to limit the pollution-induced physiological hypoxia by switching to anaerobic

metabolism, in order to compensate for this reduction of energy supply. Though an increase in the concentrations of all main anaerobic end-products was predicted, only succinate and propionate were detected after exposure to stormwater basin sediments, indicating that this worm responded to contamination with an efficient anaerobic metabolism. All these findings indicate that *L. hoffmeisteri* displays several adaptations for surviving a long-term pollution and high contaminant concentrations in heavily anthropized environments. In addition, data reported in the present study demonstrate that even though *L. hoffmeisteri* is very tolerant to urban pollutants, long-term exposures to high concentrations of urban pollutants can cause disruption in aerobic metabolism and therefore energy production. Lauer et al. (2012) showed similar results with the crab *N. granulata* exposed to an excessive concentration of copper.

Finally, this study clearly demonstrates that anaerobic end-products could be used as biomarkers to evaluate the impact of a mixture of urban pollutants on invertebrates. The proposed set of biomarkers would be clearly useful to characterize both the level and duration of contamination. Further investigations are needed to elucidate the full potential of these compounds as warning signals of toxic stress.

Acknowledgments The authors thank Prof. Eric Pattee (Université Lyon 1) for his assistance in correcting a first version of the manuscript.

Funding This study was conducted and funded within the framework of the Experimental Observatory for Urban Hydrology (OTHU; <http://www.graic.org/othu/>).

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