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Parasitism reduces oxidative stress of fish host experimentally exposed to PAHs

Noëlie Molbert [a,*], Simon Agostini [b], Fabrice Alliot [a,c], Frédéric Angelier [d], Clotilde Biard [e], Beatriz Decencière [b], Mathieu Leroux-Coyau [e], Alexis Millot [b], Cécile Ribout [d], Aurélie Goutte [a,c]

[a] Sorbonne Université, CNRS, EPHE, UMR METIS, F-75005, Paris, France
[b] CNRS ENS, UMS 3194, CEREEP–Ecotron Ile De France-Ecole Normale Supèrieure, St-Pierre-lès-Nemours 77140, France
[c] METIS, Sorbonne Université, EPHE, Université PSL, CNRS, 75005 Paris, France
[d] Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 CNRS, La Rochelle Université, F-79360, France
[e] Sorbonne Université, UPEC, Paris 7, CNRS, INRA, IRD, Institut d'Ecologie et des Sciences de l'Environnement de Paris, F-75005, Paris, France

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ABSTRACT

Some parasites are known to bioaccumulate some environmental pollutants within their host. We hypothesized that these parasites may be beneficial for their hosts in polluted environments. We experimentally increased long-term (five weeks) exposure to polycyclic aromatic hydrocarbons (PAHs, three levels: 0.1X, 1X, 10X environmental exposure) in European chubs (*Squalius cephalus*) that were naturally infected or uninfected with acanthocephalan parasites. We monitored PAHs levels in fish tissues, as well as oxidative stress, telomere length and condition indices. Although parasite infection did not significantly reduce the levels of PAHs and PAH metabolites in host tissues, host oxidative status was explained by parasitism and pollution levels. Oxidative damage increased with parasitism in fish exposed to low PAH levels (0.1X) but decreased in infected fish at higher PAH exposure (10X), thus corroborating our hypothesis. Meanwhile, antioxidant capacity did not differ in response to parasite infection nor PAHs exposure. Despite this imbalance in oxidative status, experimental increase in PAH levels did not compromise telomere length, body condition, or survival in infected and uninfected fish. This study provides the first experimental evidence that the outcome of host-parasite interactions can shift from negative to positive as pollutant exposure increases.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are an important class of persistent organic pollutants, mainly originating from anthropogenic sources (*e.g.*, industrial and domestic activities). Their continuous release on a global scale leads to widespread contamination of the environment, with common detection in aquatic ecosystems (Malaj et al., 2014). In Europe PAHs are primarily responsible for the poor chemical status of surface water bodies (European Environment Agency, 2018), exerting pressures on natural biota. Vertebrates, especially fish species, have well-developed biotransformation systems to enhance polarity and subsequent elimination of organic pollutants through bile and urine (De Maagd and Vethaak, 1998). Such processes are however important mechanisms of toxicity induced by PAHs. Their biotransformation by liver enzymes into water-soluble derivatives may lead to the formation of electrophilic intermediates, which are sometimes more noxious than their corresponding parent molecules (Wang et al., 2009) or generate reactive oxygen species (ROS) through redox cycling. In turn, these reactive molecules are capable of covalent binding to DNA and promote the formation of oxidative damages.

Adverse health effects of acute and chronic exposure to PAHs are linked to the accumulation of oxidative stress, responsible for biochemical and molecular alterations (Soltani et al., 2019). So far, fish exposure to PAHs has been associated to a wide range of health impairments, such as immunosuppression, decreased general condition, as well as adverse developmental and reproductive effects (Reynaud and Deschaux, 2006; Snyder et al., 2019). These physiological and biological changes may ultimately lead to fitness consequences, such as reduced animal's survival. More recently, human exposure to PAHs has been suggested to speed up the physiological aging process (Pavanello et al., 2020). Indeed, previous works indicate that shorter telomeres were associated with chronic PAH exposure (Pavanello et al., 2009; Hou et al.,

* Correspondence to: Sorbonne Université, CNRS, EPHE UMR METIS, 4 Place Jussieu, Paris 75252 Cedex 05, France.
E-mail address: noelie.molbert@upmc.fr (N. Molbert).

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2020) and that oxidative damage may mediate the association between chemical exposure and telomere shortening in fish (Molbert et al., 2021). Telomeres are DNA regions at the ends of chromosomes in eukaryotics that ensure genomic integrity and cell viability (Blackburn, 1991). Yet, they gradually shorten with each round of cell division, progressively resulting in cellular senescence. This natural process can however be further accelerated by external stressors, mainly oxidative stress (Reichert and Stier, 2017; Angelier et al., 2018). Composed of guanine-rich repeat sequences, telomeres are indeed particularly vulnerable to oxidative attacks (Singh et al., 2019). Because telomere length has been linked to survival and performance in various systems (Wilbourn et al., 2018, but see McLennan et al., 2019), telomere length could thus provide valuable information on the potential fitness outcome of chemical exposure in wildlife ecotoxicology.

Ubiquitous in the living world, parasites are recognized as crucial stress factors to take into account, especially when investigating the impact of environmental pollutants on fish health. Through their capacity to accumulate high levels of organic pollutants (Brázová et al., 2012; Oluoch-Otiego et al., 2016; Akinsanya et al., 2019), parasites may have a profound impact on the distribution and toxicity of chemicals in organisms, hence influencing the stress response of the host. Those parasites, mainly cestodes and acanthocephalans, are endoparasitic worms that are trophically transmitted from an intermediate host to the intestine of a vertebrate definitive host, such as fish (Kennedy, 2006). While adult parasites are usually less virulent in the final host, their establishment may lead to severe local damage and inflammatory reaction (Taraschewski, 2000), sometimes reducing growth and survival of infected organisms (Sakthivel et al., 2016; Silva-Gomes et al., 2017). Under environmental contaminations, those negative effects of parasitic infection may, however, be counterbalanced by their capacity to depurate toxicants, resulting in lower oxidative stress and enhanced survival of parasitized hosts (Gismondi et al., 2012; Sánchez et al., 2016; Morrill et al., 2019). We recently evidenced significantly lower oxidative damage in parasitized fish inhabiting chemically-altered environments (Molbert et al., 2020). Likely, the capacity of acanthocephalan parasites to accumulate pollutants, up to 700 times that in the host, may lead to reduced bioavailability of chemicals for the host and, therefore, the production of reactive species. Accordingly, *Pomphorhynchus* sp., was considered beneficial for its host under stressful chemical conditions (Weinersmith et al., 2016).

In the present study, a cross experimental design was performed on wild European chubs, naturally infected or non-infected by *Pomphorhynchus* sp. to test the benefits of parasites infection for fish hosts exposed to environmentally relevant PAH levels. By examining general biomarkers, which respond to parasite infection and pollutant exposure, we investigated the health status of the host, and in particular, how health indices (*e.g.*, oxidative status and telomere length) vary in accordance with both stress factors. We predicted that: (1) infected hosts would have lower concentrations of PAHs in their tissues than uninfected ones due to bioaccumulation within parasites, (2) exposure to PAHs would increase reactive oxygen metabolite (ROM) concentrations and decrease antioxidant capacity in fish plasma, thereby resulting in short telomeres and (3) these effects would be reduced in infected chubs given the capacity of parasites to accumulate toxicants.

2. Materials and methods

Animal care protocols were performed in accordance with laws on animal experimentation in France and Europe, and were approved by national ethics committee for animal experimentation under file number APAFIS#2018111614171570. Wild fish were captured, manipulated and transported under authorization 2019-DDT-SSE-37 delivered by the Préfecture de l'Essonne.

2.1. Capture and housing conditions

One population of European chub, *S. cephalus*, was selected on a tributary of the Marne River (49°5′42′'N, 3°40′23′'E) where the presence of intestinal parasites *Pomphorhynchus* sp. has been previously recorded (65%) and the levels of contamination is known (see Molbert et al., 2020). To control for variables susceptible to affect biomarker responses, chub were selected based on specific body length (n=160; mean±SD; 56.7±28.2 g; 16.6±2.67 cm), corresponding to immature individuals, electrofished within one week in January 2019. Fish were returned rapidly in 100-L basins filled with well-aerated river water to the CEREEP–Ecotron facilities and maintained for one week in outdoor tanks (n=5, 3 m^3) under natural conditions of temperature. Anesthetized chub (M222, 80 mg L^{-1}) were then carefully tagged with a passive integrated transponder device (8×1.4 mm FDX-B skinny tag, OREGON RFID Portland, USA) inserted intraperitoneally, weighted (±0.5 g) and measured (±0.1 cm). Tagged fish were randomly divided into sixteen 175-L tanks (80×60×42 cm) and were acclimated for 2 weeks before being exposed to PAHs (Fig. 1). Chub being gregarious, a density of 10 fish per tank was chosen to limit stressful conditions. Tanks consisted of continuously aerated systems, equipped with oxygen pumps and mechanical filters, distributed randomly within the experimental room and under a photoperiod regime fixed at a 12:12 h light/dark cycle. Water temperature was progressively warmed up to indoor conditions during the acclimation period and kept constant during the experimental activity (11.6±0.51 °C). Fish were fed twice daily with commercial fish pellets (Tetra). Half of the water was renewed every 2 days and physico-chemical parameters were monitored and held constant during the experiment (mean±SD: pH 7.93±0.09, 187±5.21 μS cm^{-1}, O_2 9.60±0.29 mg L^{-1}, saturation 88.4±2.98%).

2.2. Experimental design

A subsample of ten fish were sacrificed before the first contamination (T_0) to establish basal levels of PAHs in chub (Table S1). Five-weeks experimental PAH exposure was then performed on three groups of fifty naturally unparasitized and infected fish (a posteriori determined by dissection). Treatment groups were randomly assigned among fifteen exposure tanks, five tanks per group, to allow the evaluation of the effects of parasite infection and PAHs exposure separately and in combination on biomarker responses. The mean length and weight of chubs did not differ among tanks (ANOVA: $F_{(16,131)}$=1.715, p=0.051 and $F_{(16,131)}$=1.653, p=0.064, respectively). For each treatment group, infection status and levels of PAHs are summarized in Table 1. Pollutant concentrations were prepared from a mixed solution of 16 PAHs (see Chemical analyses for details) dissolved in cyclohexane at 10 ng μL^{-1} each, purchased from LGC standards. The exposure setup consisted of three environmentally relevant concentrations of a subset of PAHs (0.1X, 1X and 10X): 0.1X concentration at 50 ng PAHs/ g of vegetal oil, representative of the concentration found in commercial fish pellets used to feed chub during the acclimation and experimental activity; 1X concentration (500 ng PAH/g vegetal oil), representative of PAHs levels in wild invertebrate preys of chub (gammarids) captured in a river reaching good chemical status and 10X concentration (5000 ng PAHs/ g of vegetal oil), corresponding to the highest level of PAHs quantified in wild chub (Molbert, 2021). Serial dilutions in vegetal oil were carried out before each experimental exposure and were injected using a 1-mL syringe fitted with a 12-cm length of 1-mm-diameter plastic tubing into the stomach of sedated chub. Fish were carefully observed after each experimental contamination to control for oil regurgitation. The presence of oil was confirmed in their stomachs at each sampling point (ten fish were sacrificed at T_1, T_2, T_3, T_4; Fig. 1) and at the end of the procedure (T_5), therefore the dose of PAHs diluted in vegetal oil was considered the administered dose. The experimental exposures were performed on sedated fish once per week over five weeks, during which all fish were measured (±0.1 cm), weighted (±0.5 g) and checked for

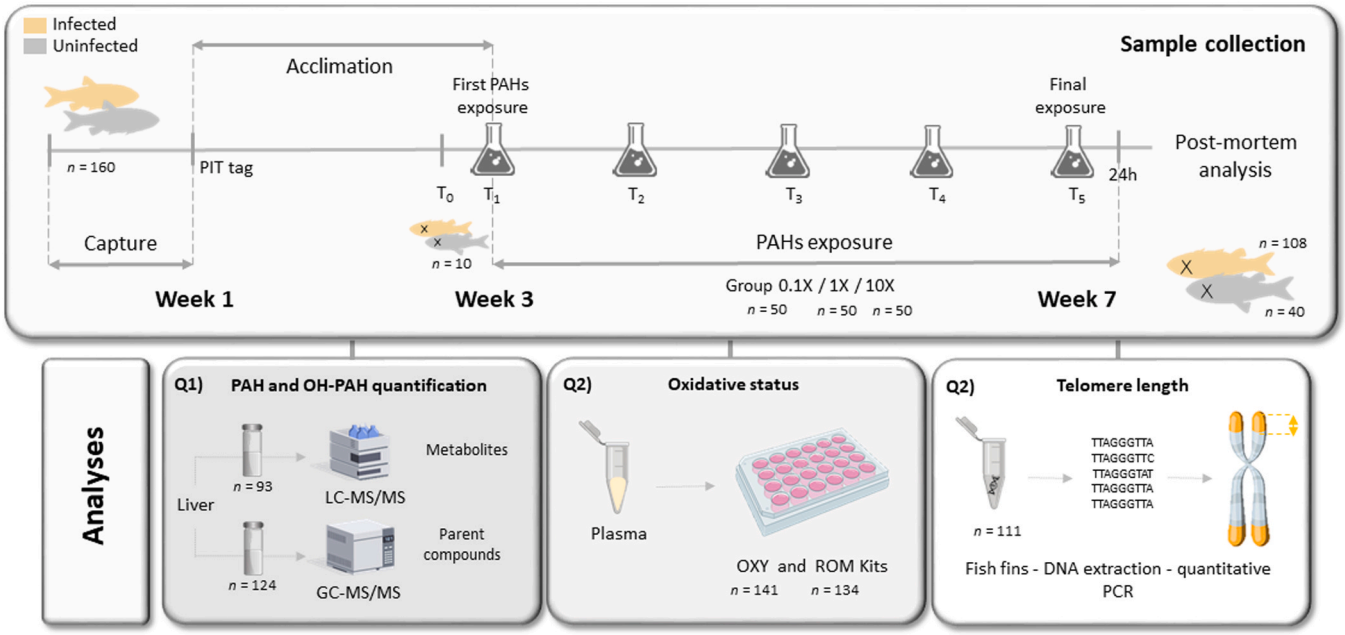


Fig. 1. Experimental design of wild chub exposed to both parasites and PAHs over five weeks. Numbers refer to sample size.

Table 1
Biometric and biologic data (mean±standard deviation [range]) of *S. cephalus* and its intestinal parasite *Pomphorhynchus* sp. among treatment group.

Group	PAH exposure[a]	Infection status	*n*	Body weight (g)	Fork length (cm)	Parasite	
						Intensity	Biomass (g)
T_0	–	Infected	**6**	79.3 (±13.9) [36.5–115]	18.8 (±1.14) [15.4–21.8]	3.33 (±0.76) [2–7]	0.04 (±0.01) [0.034– 0.046]
		Non-infected	**4**	73.5 (±13.6) [47.0–110]	18.7 (±1.03) [16.8–21.5]	-	-
0.1X	50 ng g^{-1}	Infected	**34**	54.8 (±22.1) [21.5–114]	16.7 (±2.16) [12.3–21.0]	3.47 (±2.23) [1–9]	0.04 (±0.03) [0.001–0.12]
		Non-infected	**15**	55.4 (±18.0) [31.0–79.5]	16.9 (±1.91) [13.7–19.3]	-	-
1X	500 ng g^{-1}	Infected	**41**	40.2 (±18.5) [17.5–102]	14.9 (±2.23) [11.5–20.9]	2.54 (±2.51) [1–11]	0.03 (±0.02) [0.005–0.14]
		Non-infected	**9**	49.7 (±22.9) [24.5–86.0]	15.9 (±2.49) [12.8–19.5]	-	-
10X	5000 ng g^{-1}	Infected	**33**	52.2 (±22.2) [13.0–113]	16.4 (±2.18) [13.0–22.2]	2.67 (±1.80) [1–7]	0.03 (±0.03) [0.001–0.14]
		Non-infected	**16**	57.4 (±22.4) [34.0–118]	16.8 (±2.01) [14.1–22.0]	-	-

[a] Concentrations of PAHs diluted in vegetal oil.

diseases. Before the start of the experiment, body size and weight slightly differed among groups (ANOVA: $F_{(2144)}$=8.608, p<0.001 and $F_{(2144)}$=8.501, p<0.001, respectively; Table S2), with smaller and lighter fish at 1X concentration (*post-hoc tests*: all $p \leq 0.01$). No visible health or behavioral changes were observed in chub during the acclimation and subsequent experimental periods.

2.3. Sample collection

On the last week (T_5), fish were sampled 24-h after the last PAHs exposure (Fig. 1; Varanasi et al., 1989). Blood was collected from sedated fish (MS222, 80 mg L^{-1}) *via* caudal vein puncture in 2.5 mL heparinized syringes and immediately centrifuged (10 min, 2000 *g*, 4 °C). The plasma supernatant was separated out and stored at −20 °C for subsequent oxidative damage and anti-oxidant capacity assays. Following blood sampling, fish were euthanized with an overdose of anesthetic agent (MS222, 1 g L^{-1}). Fork length (L_F; ±0.1 cm), total weight (W_T; ±0.1 g) was recorded. Pelvic fins were sampled for DNA extraction. For analysis of PAH bioaccumulation and biotransformation in fish, the liver was removed using stainless steel instruments, weighted (WL; ±0.01 g) and frozen at −20 °C until further processing. The Fulton's condition factor (K, n=148) and the hepatosomatic index (HSI, n=148) were calculated according to the following equations, to evaluate the host condition and its metabolic efficiency, respectively:

$$K=(W_T/L_F^3)\times 100$$

$$\text{HSI}=(W_L/W_T)\times 100$$

Chub intestine and body cavity were examined for the presence of acanthocephalan parasites. Whole intestine of chub were aseptically dissected. Parasites were first removed from the intestine and counted to evaluate their prevalence (the percentage of infected chub) and intensity (parasite number per infected host individuals, Bush et al., 1997). Note that during the course of the experiment, two fish out of the 150 chubs were found dead outside their respective exposure tank, because the protective netting was not secured, and were therefore excluded from the analysis.

2.4. Oxidative status

The oxidative status was evaluated by using two complementary colorimetric tests in plasma samples (n=141 for OXY and n=134 for d-ROMs because of restricted volumes). A blood-based measure of oxidative stress is indeed common in wildlife studies. First, d-ROMs test (MC003, Diacron International, Grosseto, Italy) measures the concentration of reactive oxygen metabolites (ROMs) as a measure of oxidative damage in 4 μL plasma sample. Second, to assess the antioxidant barrier of plasma, we used the OXY-Absorbent test (MC435, Diacron International, Grosseto, Italy), which quantifies non-enzymatic exogenous and endogenous antioxidants, after 1:100 dilution of 5 μL of plasma sample. Calibrations were achieved for both tests by measuring the absorbance (540 nm; iMark microplate reader, Bio rad California, USA) of a standard solution provided with the kit. Intra-assay variation for ROMs and OXY were 7.80% and 8.56%, respectively, and inter-assay variation was 7.73% (ROMs) and 10.8% (OXY). OXY concentrations were expressed as mM HOCl neutralized and ROMs as mM H_2O_2 equivalents.

2.5. Telomere length

Telomere length was determined by quantitative PCR (qPCR; BioRad CFX 96, Bio-Rad USA) according to Molbert et al. (2021), adapted for the European chub. Briefly, fin samples were digested with proteinase K and DNA was extracted using the Nucleospin Tissue Kit (Macherey-Nagel), following the manufacturer's instructions. DNA concentration and purity were assessed with a Nanodrop ND1000 spectrophotometer (Thermo Scientific) and 35 samples were excluded due to poor DNA quality (n=111). The control single-copy gene Recombination Activating Gene 1 (RAG1) was selected and amplified using specific primers (McLennan et al., 2019) designed for the European chub. Reverse and forward primer sequences for both telomeric and RAG1 genes were, respectively: Tel1b: 5′-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3′; Tel2b: 5′-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3′; RAG1-F: 5′-AGAGAGAGGGGGCTAGATGA-3′ and RAG1-R: 5′-CCATGCTTCTCGCTGACAT-3′. All qPCR runs were performed using 2.5 ng of DNA per reaction. The universal telomere primers were used at a concentration of 800 nM, and RAG1-F/RAG1-R at 300 nM. All samples were randomly distributed across the PCR plates. Amplification efficiencies reached Mean±SE: RAG1, 92.36±1.10; TEL, 95.48±3.80. The relative telomere length (expressed as *T/S* ratio) was calculated as the telomere copy number (*T*) relative to single-copy gene (*S*; RAG1), according to Cawthon (2002) and the average inter-plate variation of the *T/S* ratio values was 2.48%.

2.6. Chemical analyses

A total of 16 parent compounds of PAH (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3–cd]pyrene, dibenzo[a,h]anthracene, and benzo[ghi]perylene) and 12 hydroxylated metabolites of PAHs (OH-PAH; 1–hydroxynaphthalene, 2–hydroxynaphthalene, 2–hydroxyfluorene, 3- hydroxyfluorene, 9- hydroxyfluorene, 1–hydroxyphenanthrene, 2– hydroxyphenanthrene, 3– hydroxyphenanthrene, 4– hydroxyphenanthrene, 1–hydroxypyrene, 6–hydroxychrysene, and 3–hydroxybenzo[a]pyrene), listed as priority pollutants by the United States Environmental Protection Agency, were quantified in fish liver (n=124 for parent molecules and n=93 for their metabolites), following previously published protocols (Molbert et al., 2019). For each compound, highly linear responses were obtained (R^2>0.98) over the concentration ranges tested (from 12.5 ng mL^{-1} to 5000 ng mL^{-1} and from 5 ng mL^{-1} to 1250 ng mL^{-1} for parent compounds and some of their metabolites, respectively). Recoveries ranged from 52% to 103% for PAHs and from 31% to 119% for OH-PAHs, with a mean variability of 9% and 22%, respectively. Metabolites were quantified in fewer individuals since some of them did not yield sufficient biological material to carry out chemical analyses. Approximately 0.1 g of freeze-dried and powdered fish liver was used for quantitative analyses of pollutants. For detailed description of the analytical methods, refer to Molbert et al. (2019). Analyte concentrations below the limit of quantification (LOQ) value were replaced by half of the LOQ. Concentrations of parent molecules (∑16 PAHs) and their metabolites (∑12 OH-PAHs) were expressed as ng g^{-1} of dry weight (dtw).

2.7. Statistical analyses

All statistical models were performed in R v. 3.3.2 software (R Core Team, 2016). Analyses were performed using the *lmer* statistical procedure available in the *lme4* and *lmerTest* packages for linear mixed models (LMM, Pinheiro and Bates, 2000; Kuznetsova et al., 2017). Variance components were estimated using a restricted maximum likelihood (REML) function and all models had normal error structures. Data are presented as mean ±standard error (SE) and a significance level of $\alpha<0.05$ was used for all tests.

2.7.1. Effect of parasitism on the pollutant burden in chub

We tested whether parasite infection may reduce host contamination, with concentrations of ∑16 PAHs or their metabolites (∑12 OH-PAHs) as dependent variables, log-transformed to achieve a normal distribution. Initial models included as fixed factors parasite infection status (two levels factor: infected or non-infected), treatment group (three levels factor: Low, Medium, High), and their first-order interaction. Tank ID was treated as a random effect. We used a backward elimination process to exclude non-significant variables, starting with interactions, to produce minimum adequate models, providing that this resulted in a reduction of the bias-adjusted Akaïke's information criterion (AICc) score for small-sample size. When interactions were significant, we used contrast post-hoc analyses with adjusted p-values to analyze differences between groups (*emmeans* package; Lenth et al., 2017). Pearson's test followed by linear models were applied to evaluate the correlation between levels of PAHs in fish liver and parasite biomass.

2.7.2. Effect of PAH exposure and parasitism on biomarkers and health indices

Telomere length, oxidative status (ROM and OXY) and condition indices (changes in the general condition over the experiment ($\Delta_{T0\text{-}T5}$ Fulton's *K*), and end-point HSI and *K*) were used as dependent variables. Telomere attrition is linked to normal aging in fish (Hatakeyama et al., 2016). Given that telomeres shortened with fish size in this study (LM: $F_{(1111)}$=6.109, p=0.014; β±s.e.=−0.036±0.014), telomere length was first corrected with body size to account for differences between treatment groups. Residuals were then used as a dependent variable given their normality (Shapiro-Wilk: p=0.107) and homogeneity of variances (Breusch-Pagan: p=0.311). All initial models included as fixed factors the effects of treatment group, parasite infection and their first-order interaction. Tank ID was treated as a random effect. Results are presented as mean parameter estimates ±standard error (β±SE). We performed diagnostic plots and Shapiro normality tests on residuals to check model assumptions. Additionally, we checked whether telomere length (T/S ratio) was related to oxidative status with Spearman correlations, as the oxidative status proved to be non-normally distributed.

3. Results

At the end of the experiment, the prevalence of intestinal parasites was 69%, 80% and 67% in fish exposed to 0.1X, 1X and 10X, respectively, which did not differ among treatment groups (X^2=3.171, d.f.=2, p=0.204). The intensity of intestinal parasite did not differ among treatment groups (Poisson GLM: $F_{(2105)}$=1.738, p=0.181), with a mean number of *Pomphorhynchus* sp. (±SE) per infected fish of 3.47±0.32, 2.54±0.35 and 2.67±0.26 at 0.1X, 1X and 10X, respectively.

3.1. PAHs and OH-PAHs in liver

Analysis of fish tissues at the start of the experiment (T_0) revealed high levels of PAHs in chub muscle (n=10; 399±78.4 ng g^{-1} dwt) and liver (n=5; 784±159 ng g^{-1} dwt), ten times higher than the levels from a previous field study conducted at the same sampling site (n=6; 76±27.0 ng g^{-1} dwt in liver; Molbert et al., 2020). Mean levels of PAHs (±SE) at T_0 did not significantly differ between infected (593±131 ng g^{-1} dwt, liver) and uninfected chub (1072±251 ng g^{-1} dwt, liver) (Fisher-Pitman permutation test: p=0.55). After five-week exposure, PAHs levels were significantly influenced by the interaction between infection status and treatment group (LMM: $F_{(2111.6)}$=4.022, p=0.020; Supplementary Material Table S3; Fig. 2A) with higher levels of PAHs in the liver of infected chub exposed to 10X compared to infected fish exposed to 0.1X (*post hoc test*: p=0.032, Table S4). Although infected and uninfected host did not differ in their pollutant load, levels of PAHs in parasitized chub decreased with increasing parasite biomass (Pearson correlation: r_p=−0.24, p=0.028). After 5 weeks of exposure, levels of liver metabolites (∑12 OH-PAHs) varied among treatment groups (LMM: $F_{(2,14.71)}$=12.08, p=0.001;Table S1), with significantly higher levels in fish exposed to 10X compared to 0.1X and 1X (Table S4, Fig. 2B). There was a positive correlation between parent PAHs and the levels of liver metabolites (Spearman correlation: r_s=0.25, p=0.029).

3.2. Biomarker responses to parasite infection and PAHs contamination

3.2.1. Condition indices

Throughout the experiment, changes in the general condition ($\Delta_{T0\text{-}T5}$ Fulton) were negatively affected by increasing PAHs exposure (treatment, LMM: $F_{(2144)}$=4.084, p=0.019) but not by parasite infection (LMM: $F_{(2144)}$=0.702, p=0.403). The condition of chubs exposed to 0.1X significantly increased over five weeks (mean±SE, 0.035±0.009) compared to 1X and 10X [0.003±0.009 and 0.007±0.008, respectively; *post hoc tests*: p=0.009 (0.1X vs. 1X), p=0.025 (0.1X vs. 10X); Fig. 3]. At the end of the experiment, variation in Fulton's body condition (*K*) was not significantly explained by any variable (Table S5, Fig. 4A). The hepatosomatic index (HSI) did not differ between infected and uninfected chub (LMM: $F_{(1144)}$=3.093, p=0.08; Tables S5 and S6) but differed among treatment groups (LMM: $F_{(2144)}$=3.645, p=0.028), with higher HSI at 10X concentration [*post hoc test*: p=0.048 (10X vs. 0.1X), Fig. 4B].

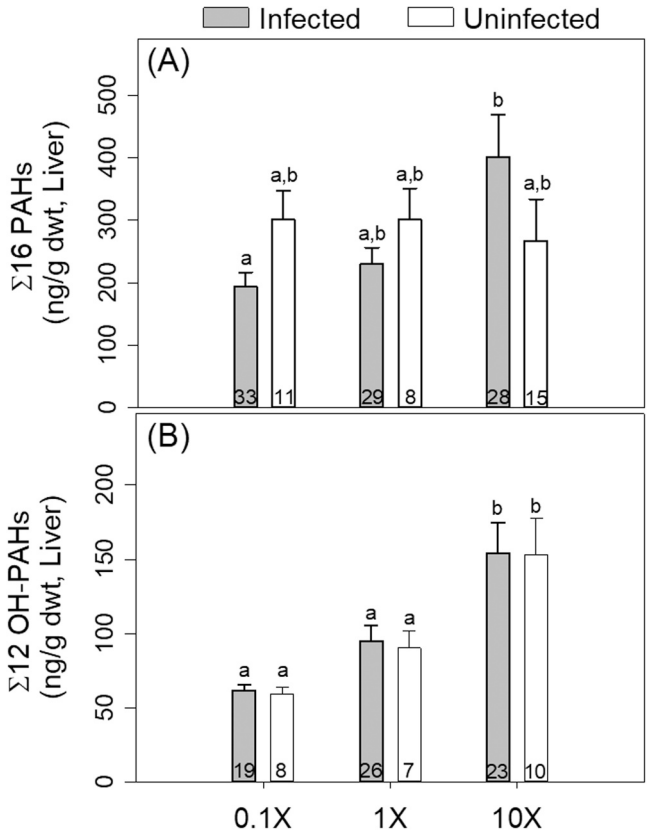


Fig. 2. Levels of **(A)** ∑ 16 PAHs and **(B)** ∑ 12 OH-PAHs in chub (ng g^{-1} dry weight, Liver) among treatment group (levels of PAH exposure). Numbers represent sample size and error bars represent standard error. Significant differences (*post-hoc tests*: p<0.05) between groups are indicated by different letters [a,b]. Data are expressed as the mean±standard error.

3.2.2. Oxidative status

Variation in total plasma antioxidant capacity was not significantly explained by any variable (Table S5, Fig. 4C). At the end of the experiment, variation in ROM concentrations in chub plasma was explained by the interaction between infection status and treatment group (LMM: $F_{(2125.8)}$=6.321, p=0.002; Tables S5 and S6). Parasitized chub exposed to 0.1X displayed 41% higher ROM concentrations (*post hoc test*: p=0.007; Fig. 4D) than uninfected ones, whereas ROM concentrations in chub exposed to 10X were 39% lower in infected individuals compared to uninfected ones (*post hoc test*: p=0.021). In addition, ROM concentrations were significantly higher in parasitized chubs exposed to 0.1X compared to 10X and to a lesser extent 1X [*post hoc tests*: p=0.025 (0.1X vs. 10X), p=0.054 (0.1X vs. 1X)]. At 1X concentration, no difference in ROM levels was observed between chubs infected or not by acanthocephalan parasites (*post hoc test*: p=0.559).

3.2.3. Telomere length

Telomere length did not significantly differ between 0.1X, 1X and 10X (T/S ratio: 1.12±0.05, 1.16±0.06 and 1.04±0.05, respectively; Table S5, Fig. 4E) and was not related to the infection status of chubs [T/S ratio: 1.14±0.04 (infected) and 1.03±0.05 (uninfected); Table S5]. Additionally, whatever the treatment group, there was no correlation between telomere length and total antioxidant capacity of chub (Pearson

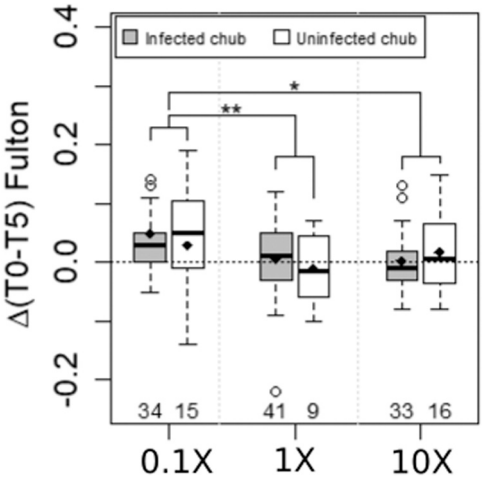


Fig. 3. Variation of fish host general condition over five weeks of experimental PAH exposure ($\Delta_{T0\text{-}T5}$ Fulton), among treatment groups (levels of PAH exposure) and for infected (gray) versus uninfected hosts (white). The dotted line indicates no change in body condition. A value below the dotted line means a declining general condition of chubs after 5-week experimental exposure, and vice versa. Box- and whisker plots show high, lox and median values (black horizontal line); black dots represent mean values; lower and upper edges of each box denote first and third quartiles; vertical dashed lines denote the most extreme values; open dot represents outliers. Numbers below each box are sample sizes. Asterisks indicate significant effect of treatment (*: p<0.05; **: p<0.001).

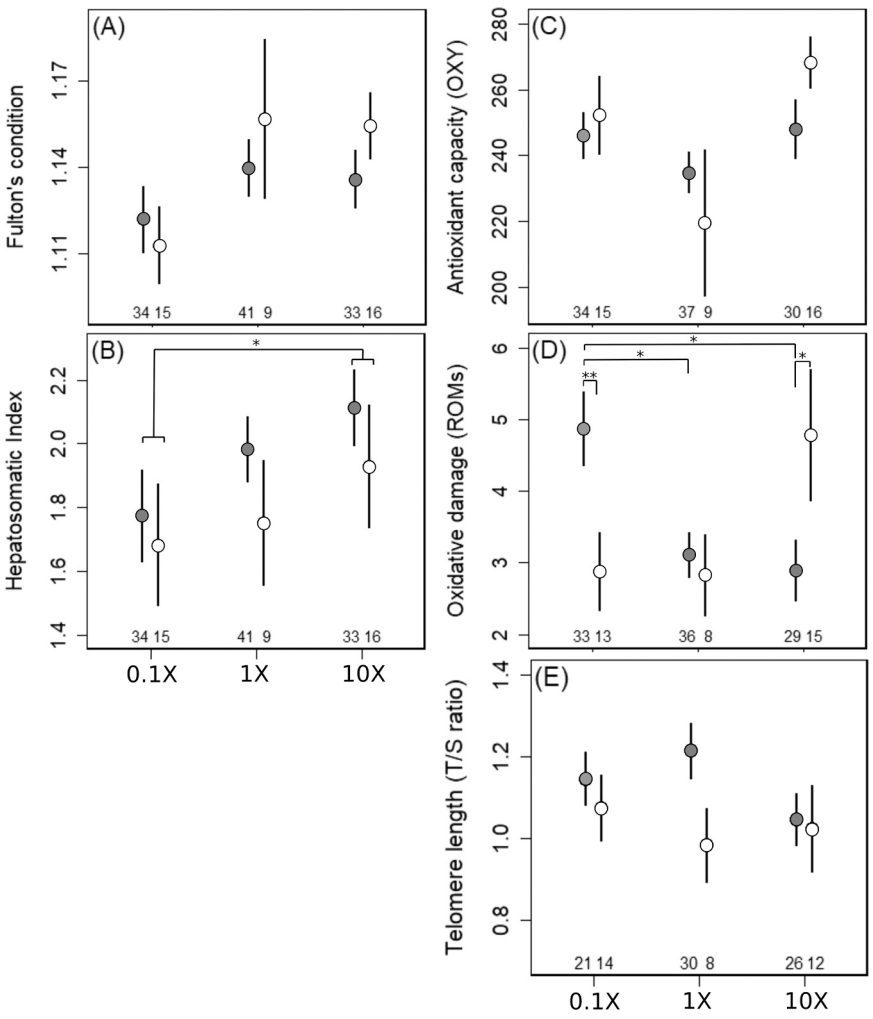


Fig. 4. Effect of both parasite infection (gray: infected, white: uninfected) and experimental level of PAH exposure on chub (**A**) body condition, (**B**) hepatosomatic index, (**C**) antioxidant capacity (OXY, mM HOCl neutralized), (**D**) oxidative damage (ROMs, mM H_2O_2 equivalents) and (**E**) telomere length (T/S ratio) after five-week exposure. Data are expressed as the mean ±standard error. Numbers represent sample size. Asterisks indicate significant effect of treatment (*: $p<0.05$; **: $p<0.001$).

correlation: $r_p \leqslant 0.08$, all $p \geqslant 0.447$) nor between telomere length and ROM concentrations (Spearman correlation: $r_s \leqslant 0.10$, all $p \geqslant 0.572$).

4. Discussion

We experimentally investigated the impact of chemical pollution on host-parasite interactions, by exposing fish naturally infected or not by acanthocephalan parasites to environmental relevant levels of PAH.

Our first objective was to test whether parasitized chubs exhibited lower PAH levels compared to uninfected fish, as a result of bioaccumulation in acanthocephalans. We observed a non-significant trend towards a lower level of PAHs in infected chub compared to uninfected ones. Former studies evidenced the capacity of intestinal parasites to accumulate high levels of PAHs (Molbert et al., 2020; Soler-Jiménez et al., 2020) and to reduce the pollutant load of their fish host (Vidal-Martínez et al., 2003; Brázová et al., 2012). Our results support the general trend reported by other authors of a decrease in pollutant load in hosts when parasite biomass increases (Sures, 2002). Although chubs were weekly exposed to doses of PAHs ranging from 50 to 5000 ng g^{-1}, few significant differences in pollutant load were observed among treatment groups, probably because fish are able to convert up to 99% of PAHs to metabolites within 24 h of the uptake (Varanasi et al., 1989). PAH metabolites were indeed detected in fish tissue, with the highest levels in the high treatment group (10X). Additionally, a positive correlation between parent PAH and liver metabolite (OH-PAH) concentrations, ensures that PAHs-contaminated oil was absorbed and biotransformed after exposure. This is further supported by the liver enlargement observed in the high exposure group, which usually reflects enzyme induction (*i.e.,* an increase in the rate of hepatic metabolism, as measured by the hepatosomatic index). Accordingly, liver hypertrophy in fish species has been associated with PAH chronic exposure or polluted environments (Everaarts et al., 1993; Larno et al., 2001).

Interestingly, no mortality was recorded over five-week exposure and no external or internal lesions were observed, whether fish hosts were parasitized or not. In addition, very few PAHs or parasite-related effects were noted on the Fulton's condition factor (*K*) and hepatosomatic index (HSI) at the end of the experiment. The Fulton's condition factor is however considered as a long term parameter integrating the general well-being of the fish at the scale of weeks or months (Suthers, 2000). Throughout the experiment (5 weeks), changes in the general condition ($\Delta_{T0\text{-}T5}$ Fulton) were negatively affected by increasing PAH exposure. This could be explained by a potential change in energy allocation in response to contaminant exposure, preferentially for resistance to chemical stress to the detriment of growth (Lenhardt et al., 2009; Kerambrun et al., 2013). Alternatively, previous studies have attributed the decrease in Fulton's condition with contaminant exposure to a decline in feeding activity or a major reduction in the ability to assimilate and/or convert food to energy. This assumption was not fully supported in the present study. First, fish were hand-fed to monitor feeding activity of each individuals and there was no uneaten food left after a feeding. Second, liver enlargement demonstrates the metabolic efficiency of fish. However, we cannot rule out the possibility that assimilation may have been affected by contaminant exposure. Overall, those results suggest that parasitic infection did not generate higher

energetic demands nor compromise the condition of the host, which concur well with previous studies (Hursky and Pietrock, 2015; Lagrue and Poulin, 2015; Molbert et al., 2020). The apparent lack of pathological effects at higher biological scales may be linked to the relatively low number (from 1 to 11 per fish) of *Pomphorhynchus* sp. in the fish host. Indeed, intestinal parasite abundance or biomass and their depth of penetration into the host tissue are the main factors known to cause damage to key host organs and alter body condition (Taraschewski, 2000; Maceda-Veiga et al., 2016).

The main goal of this study was to test whether parasitism may be beneficial for their host in polluted environment. At a lower biological scale, exposure to both parasite infection and PAHs had an effect on the oxidative status of the host. Indeed, plasmatic ROM concentrations significantly varied among treatments groups in interaction with infection status. The d-ROMs test mainly measures hydroperoxides, which derive from the oxidation of biomolecular substrates and act as precursors of end-products of lipid peroxidation (Beaulieu and Costantini, 2014). Hydroperoxides are thus perceived as the earliest markers of oxidative damage in a biological system. Secondly, total plasma antioxidant activity (OXY), did not significantly differ among treatment groups, nor between infected and parasitized-free individuals. Over the course of the experiment, fish were fed with commercial pellets enriched in vitamins involved in the redox process. This feeding protocol might have buffered the expected effect of pollutant exposure on antioxidant defences given that fish were not constrained with regard to antioxidant availability. Overall, our results indicate a negative effect of the intestinal worm on its host exposed to low pollutant levels. This likely reflects the direct cost of infection by *Pomphorhynchus* sp. on its fish host through the induction of local inflammatory reactions (Dezfuli et al., 2011). Conversely, being parasitized had a positive effect on hosts exposed to increasing levels of contaminants. This advantageous effect of parasites on host physiology (oxidative status), has been previously attributed to the capacity of intestinal parasites to accumulate toxicants and depurate their host (Morrill et al., 2019; Molbert et al., 2020), therefore reducing electrophilic intermediates generated through the metabolization of parent compounds. In chemically-altered conditions, the ability of intestinal parasite to act as internal sinks for pollutants could therefore outweigh the direct cost of parasitism. The outcome of host-parasite interactions may thus be context dependent, with potential benefits in polluted environments (Weinersmith and Earley, 2016). While the combined effect of parasitism and pollutant exposure affected the oxidative status of chub, no statistically significant effects were detected on the telomere length. Although telomeres are highly sensitive to oxidative damage, which can accelerate telomere shortening (Reichert and Stier, 2017; but see Boonekamp et al., 2017) we detected no associations between those two physiological markers. However, considering the brief exposure to environmental stressors (5 weeks), we cannot rule out that a significant relationship might have arisen over a longer experimental period. Additionally, telomeres were only measured at the end of the experiment and telomere length was highly variable between individuals. As a result, the large inter-individual variability may have blurred the potential effect of PAHs exposure and parasite infection on telomere length. For that purpose, studies should focused on the effect of both chemical exposure and parasite infection on telomere dynamics in wild animals.

5. Conclusion

In the present study, we experimentally tested whether acanthocephalan parasites *Pomphorhynchus* sp. are beneficial to their final host under chemical exposure. While parasite infection did not reduce the pollutant load of their fish host, nor affected biotransformation processes, infection was associated with changes in the oxidative status. Moreover, levels of PAHs in infected chubs decreased with increasing parasite load, and the experimental design allows us to demonstrate a shift in parasite effect on the physiology of its host, from negative to positive, as chemical exposure increases. Our results make some useful and original contributions to the understanding of pollutant-induced modulation of interspecific interactions, as intestinal parasites can provide benefits for their hosts under chemically stressful conditions.

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CRediT authorship Contribution Statement

A.G. acquired the funding. A.G. and N.M. conceived the idea and designed the methodology. A.G., F.Al. and N.M. captured the fish. A.M, S.A., B.D. and N.M provided animal care. N.M. carried out chemical analysis and chromatographic acquisition, ran statistical analyses and drafted the manuscript. M.L.-C., N.M. and C.B. evaluated the oxidative status. F.A. and C.R. performed telomere assays. All authors contributed critically to the manuscript draft and gave final accordance for publication.

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.2021.112322.

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