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# Potential Benefits of Acanthocephalan Parasites for Chub Hosts in Polluted Environments

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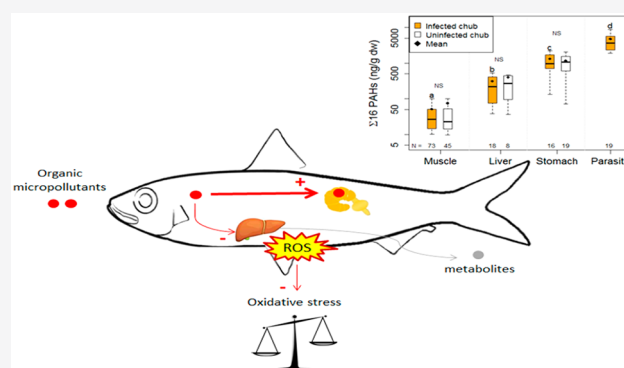


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**ABSTRACT:** Some parasites are expected to have beneficial impacts on wild populations in polluted environments because of their bioaccumulation potential of pollutants from their hosts. The fate of organic micropollutants in host–parasite systems and the combined effect of parasitism and pollution were investigated in chub *Squalius cephalus*, a freshwater fish, infected ( $n = 73$ ) or uninfected ( $n = 45$ ) by acanthocephalan parasites *Pomphorhynchus* sp. from differently contaminated riverine sites. Several ubiquitous pollutants (polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl-ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), phthalates, insecticides, pyrethroids, and *N,N*-diethyl-meta-toluamide (DEET)) and some of their metabolites were characterized for the first time in parasites and various fish matrices (muscle, liver, and stomach content). Most organic pollutants reached higher levels in parasites than in chub matrices. In contrast, metabolite levels were lower in parasite tissues compared to fish matrices. Infected and uninfected chub exhibited no significant differences in their pollutant load. Body condition, organo-somatic indices, and immunity were not affected by parasitism, and few correlations were found with chemical pollution. Interestingly, infected chub exhibited lower oxidative damage compared to uninfected fish, irrespective of their pollutant load. In light of these results, this correlative study supports the hypothesis that acanthocephalan parasites could bring benefits to their hosts to cope with organic pollution.



## INTRODUCTION

Parasitic interactions can switch to mutualistic ones, and vice versa, under stressful biotic<sup>1</sup> and environmental<sup>2–4</sup> conditions. Among the multiple factors investigated, the effect of chemical pollution on species interaction remains poorly investigated.<sup>5</sup> In aquatic environments, such an effect is however of primary importance given the direct proximity to contamination sources (poor wastewater treatment, stormwater runoff, leaching from green spaces and agriculture, accidental discharge; see the work of Pierce et al.<sup>6</sup>).

Acanthocephalan parasites are endoparasitic worms that are transmitted from an arthropod intermediate host to the intestine of a vertebrate definitive host.<sup>7</sup> These parasites are known to negatively alter the physiology of fish<sup>8</sup> and influence life-history traits such as reproduction<sup>9</sup> and behavior.<sup>10</sup> On the other hand, acanthocephalan parasites could act as toxicant sinks and reduce the pollutant load of their host.<sup>11</sup> Intestinal parasites, particularly *Pomphorhynchus* sp., are able to concentrate environmental pollutants, sometimes at orders of magnitude higher than in their host tissues.<sup>12–15</sup> Up to now, studies have mainly focused on trace metals and more recently on polychlorinated biphenyls (PCBs),<sup>16</sup> a family of persistent

pollutants no longer produced or used. To date, very few studies have considered the effects of emerging organic pollutants<sup>17,18</sup> and none of them have simultaneously evaluated the fate and physiological consequences of these pollutants in host–parasite systems. Such widespread and chronically released chemicals, including phthalates, polycyclic aromatic hydrocarbons (PAH), and some pesticides, raise concerns about freshwater ecosystems.<sup>19</sup> In addition, their metabolization leads to the formation of toxic byproducts that are sometimes even more noxious than the parent pollutants.<sup>20</sup>

Studying the fate of pollutants in host–parasite systems is a challenging task since it requires taking into account biological and chemical factors involved in the ability of each organism to accumulate, metabolize, and excrete organic pollutants. First, chemical uptake and its accumulation inside organisms may

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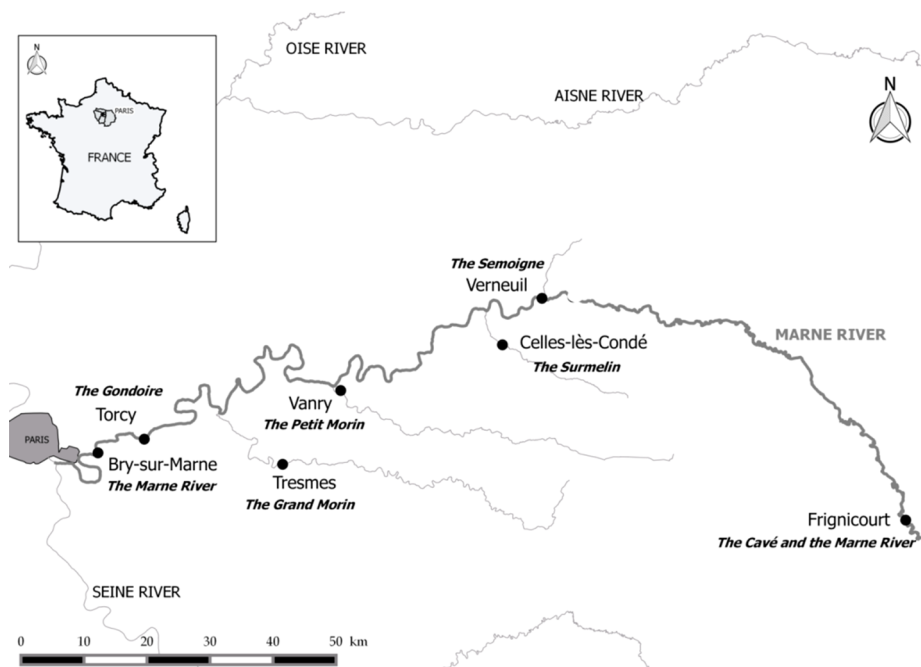


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**Figure 1.** Sampling sites on the Marne River (France) and its tributaries (Created using QGIS 3.4.12 software on French geoportal IGN-F layers).

vary with the trophic level, length/age, and lipid contents for hydrophobic molecules (i.e., octanol–water partition coefficient:  $\log K_{OW}$ ).<sup>21,22</sup> Second, vertebrates and invertebrates differ strongly in their ability to metabolize xenobiotics,<sup>23</sup> so that the pollutant's susceptibility to metabolic transformation ( $\log k_M$ ) is expected to affect the fate of pollutant in host–parasite systems. Lastly, the properties of pollutants can affect their distribution inside the studied system.<sup>24</sup> Acanthocephalan parasites lack a gastrointestinal tract, so that food assimilation mainly occurs through passive mechanisms.<sup>25</sup> This physiological trait is likely to favor smaller and more water-soluble molecules,<sup>26,24</sup> which are able to cross the parasite tegument.

By interfering with the bioaccumulation of various pollutants, parasites may have a profound impact on the host resistance to pollution. However, interactions between hosts and parasites in polluted environments are complex. Several studies have highlighted that lethal and sublethal effects of chemicals can be exacerbated by parasitism, through reduced survival,<sup>27</sup> body conditions,<sup>28</sup> and enhanced oxidative stress.<sup>29</sup> On the other hand, cestode infection has been shown to reduce intermediate host mortality across a range of arsenic concentrations.<sup>30</sup> Similarly, decreased survival with increasing lead (Pb) levels was observed in common eiders (*Somateria mollissima*) treated with an antiparasite drug,<sup>5</sup> suggesting a protective effect of parasites in Pb-exposed individuals. General biomarkers are known to respond to a variety of stressors, natural or anthropogenic,<sup>31</sup> being thus appropriate to investigate the response of host–parasite systems to pollutant exposure.

At the physiological level, oxidative stress is defined as a dynamic imbalance between the production of reactive oxygen species (ROS) and their detoxification through enzymatic and nonenzymatic defenses, causing damage to cellular components.<sup>32</sup> In the context of the effects of parasitism in polluted environments, ROS are generated by inflammation (due to parasite infection) and metabolic processes (biotransformation of pollutants). In addition, both chemical exposure and

parasite infection can negatively alter antioxidant defenses,<sup>33,34</sup> which afford protection against ROS. As a consequence, both stressors are expected to have interactive effects on the oxidative status. Enzyme systems related to the innate immunity (e.g., lysozyme, peroxidase) are also used as biomarkers of nonspecific stress responses. For instance, organic pollution has been linked to reduce immunocompetence of the host, promoting parasitic infection and diseases.<sup>35</sup> Yet, studies have reported contrasting results on the joint effect of pollution and parasitism on immunity.<sup>36,37</sup>

At a higher biological level, organ-somatic indices and body condition may provide valuable information on the potential costs and benefits of parasites in contaminated environments. First, pollutants have been known to either stimulate or weaken the feeding behavior of fish,<sup>38</sup> thus impacting their energetic status. In addition, parasites can have neutral, negative, or positive effects on the host body condition,<sup>39–41</sup> by depleting host resources or by enhancing host lipid content.<sup>30</sup> The combined effects of parasitism and pollution on fish health are therefore still poorly understood, especially in natural field conditions.

We focused on a freshwater fish, the European chub, *Squalius cephalus*, and its intestinal acanthocephalan parasite *Pomphorhynchus* sp., both are widespread and abundant species in Europe.<sup>42</sup> Living up to 20 years and tolerant to pollution, chub are good ecotoxicological models prone to accumulate toxicants.<sup>43</sup> In the present study, we aimed to (i) test whether acanthocephalan parasites accumulated organic pollutants from their host, and preferentially those with high metabolic transformation rate ( $\log k_M$ ), high solubility, and low molecular weight, (ii) test whether the pollutant load was lower in parasitized fish, and (iii) evaluate the potential benefits of parasite infection in chub, considering their pollutant load, at the molecular/cellular (oxidative status and immune biomarkers) and organ/organismal levels (hepato-somatic index and body condition).

## MATERIALS AND METHODS

**Ethics Statement.** Fish sampling was conducted in accordance with the relevant national and European guidelines (L436-9, EN14011). The authorization for the scientific fish capture was delivered by local administration authorities (Departmental Direction of Territories of Seine-et-Marne).

**Study Area and Sample Collection.** A total of 118 specimens of European chub (*Squalius cephalus*), from 8.80 to 41.5 cm in length (mean  $\pm$  SD:  $19.9 \pm 7.16$  cm), were electrofished in September 2016, within 12 days to reduce temporal effects. Samplings were conducted on seven stations from the Marne River and its tributaries (Figure 1), France, along a 290-km-long stretch of the river. Sites were selected based on their proximity to emission sources of organic pollutants:<sup>44</sup> from agricultural (for organochlorine pesticides (OCPs)) to urban (phthalates, PAHs, and pyrethroids)/industrial (PCBs and flame retardants: PBDE) areas. Fish from different sampling sites were considered distinct populations given their relatively short-range movements.<sup>45</sup>

Blood was collected from sedated fish (tricaine methanesulfonate: MS222,  $80 \text{ mg} \cdot \text{L}^{-1}$ ) via caudal vein puncture in 2.5 mL heparinized syringes and 23-gauge needles. Samples were centrifuged for 5 min at 600 g, and plasma was separated from blood cells for biomarker analyses (oxidative status and immunity). Fish were then sacrificed in 1 g/L MS222 solution. Total length ( $L_T$ ,  $\pm 0.1$  cm) and body weight ( $W_T$ ,  $\pm 0.1$  g) were recorded prior to tissue sampling. The Fulton's condition factor [ $K = (W_T/L_T^3) \times 100$ ] was calculated to assess the host condition ( $n = 118$ ). Muscle, liver, and stomach contents were dissected using stainless steel instruments. The liver was weighed ( $W_L$ ,  $\pm 0.01$  g) for each fish to compute the hepatosomatic index [ $HSI = (W_L/W_T) \times 100$ ] and thus evaluate the host energetic status and/or metabolic efficiency.<sup>46</sup> Biological samples were stored in polycarbonate tubes to limit phthalate contamination and frozen at  $-20^\circ\text{C}$  until further treatment.

**Intestinal Parasites.** Chub intestine and body cavity were examined for the presence of acanthocephalan parasites. Without molecular-based identification, parasites are reported under the genus *Pomphorhynchus*. As expected, over half of the fish were infected ( $n = 73$ ; mean total prevalence 61.9%), and all parasites were found within the host intestine. Acanthocephalans were carefully removed from the chub intestine and all parasites from the same host individual were pooled for chemical analysis. From 0.99 to 500 mg of freeze-dried parasite biomass was recorded per fish. Only 19 pools yielded sufficient biological material for chemical analyses.

**Age Determination.** Scales were collected from each specimen above the lateral line for age determination. All scales were stored dry until the age determination was achieved by counting the number of annuli on the scales.<sup>47</sup> Regenerated scales were avoided, and the others were observed under a Leica M205C Stereomicroscope. As it is difficult to estimate the age of cyprinid fishes older than 10 years, age determination was independently made by two different operators to limit interpretation bias.

**$\delta^{15}\text{N}$  Analysis.** Isotopic measurements were performed on 0.2–0.25 mg of freeze-dried and powdered muscle samples ( $n = 113$ ; some samples were lost because of combustion issues). Samples were analyzed by means of a Flash HT elemental analyzer (ThermoFisher) coupled to a Delta V mass spectrometer (ThermoFisher) at the IRD laboratory in Bondy

(France). Isotope ratios are expressed in parts per thousand (‰) compared with standard values of atmospheric nitrogen as  $\delta^{15}\text{N} \text{ ‰} = [(R_{\text{ech}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R = {}^{15}\text{N}/{}^{14}\text{N}$ . The analytical precision was 0.7‰, and we used  $\delta^{15}\text{N}$  to estimate trophic position.

**Biomarkers of Innate Immunity.** The enzymatic activities of peroxidase and lysozyme are two commonly used biochemical markers to evaluate the fish innate immune responses.<sup>48</sup> Due to their lytic activity, lysozymes exert protective effects against microbial invasion and peroxidase plays a role in the oxidative response against pathogens.

Peroxidase activity was determined according to Quade and Roth<sup>49</sup> at Bordeaux Sciences Agro (Gradignan, France). Briefly, a 96-well plate was filled with 5  $\mu\text{L}$  of fish plasma in 400  $\mu\text{L}$  of HBBS (Hank's balanced salt solution) buffer. The reaction mixture was added to the microplate and consisted of 100  $\mu\text{L}$  of  $\text{NaH}_2\text{PO}_4$  (4.5 mL)/citric acid buffer and 0.5 mL of TMB (3,3',5,5'-tetramethylbenzidine) solution as the chromogen substrate. After 2 min, the reaction was stopped with 50  $\mu\text{L}$  of sulfuric acid ( $\text{H}_2\text{SO}_4$ , 2 M). The absorbance was recorded at 450 nm.

Following the procedure described by Ellis,<sup>50</sup> lysozyme activity was measured using fish plasma (15  $\mu\text{L}$ ) and 150  $\mu\text{L}$  of a solution of *Micrococcus lysodeikticus* suspension (20 mg  $\text{mL}^{-1}$ ). The change in turbidity was measured at 450 nm every 5 min within 40 min. The lysozyme and peroxidase activity was expressed in units of inverse milliliters. As a few samples were too small to be used in both assays, sample sizes differed slightly between lysozyme ( $n = 69$ ) and peroxidase ( $n = 80$ ) analysis.

**Biomarkers of Oxidative Status.** The oxidative status was evaluated by using two complementary colorimetric tests in plasma samples ( $n = 99$  for OXY and  $n = 90$  for d-ROMs because of hemolyzed samples and restricted volumes). First, d-ROMs test (MC003, Diacron International, Grosseto, Italy) measures the concentration of reactive oxygen metabolites (ROMs) as a measure of oxidative damage. Hydroperoxides are the main molecules measured by the d-ROMs,<sup>51</sup> which derive from the oxidation of biomolecular substrates and act as precursors of end-products of lipid peroxidation.<sup>52</sup> Second, to assess the antioxidant barrier of plasma, we used the OXY-Absorbent test (MC435, Diacron International, Grosseto, Italy), which quantifies nonenzymatic exogenous and endogenous antioxidants. Analyses were run following Vaugoyeau et al.,<sup>53</sup> using 4  $\mu\text{L}$  (d-ROMs) and 5  $\mu\text{L}$  (OXY) 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 10.9% and 6.2%, respectively, and interassay variation was 11.6% (ROMs) and 7.4% (OXY). These coefficients were evaluated by measuring each sample twice (intra-assay) and three replicated subsamples (interassay) per plate. OXY concentrations were expressed as millimoles of HOCl neutralized and ROMs as millimoles of  $\text{H}_2\text{O}_2$  equivalents.

**Chemical Analysis and Lipid Contents.** The analytical methods used are described in the work of Molbert et al.<sup>44</sup> A total of 48 parent compounds (16 polycyclic aromatic hydrocarbons (PAHs); 7 phthalate esters; 7 pyrethroids; DEET; 4 organochlorine pesticides (OCPs); 7 polychlorinated biphenyls (PCBs); 6 polybrominated diphenyl ethers (PBDEs)) and some of their metabolites (11 hydroxylated PAHs (OH-PAHs), 9 phthalate monoesters; 4 byproducts of



**Table 1.** Ratios of Pollutant Concentrations (ng g<sup>-1</sup> of dry wt) in *Pomphorhynchus* sp. Relative to That in Host Muscle (*n* = 19), Liver (*n* = 9), and Stomach Content (*n* = 12)<sup>a</sup>

compounds	$C_{[\text{parasite}]} / C_{[\text{chub muscle}]}$	$C_{[\text{parasite}]} / C_{[\text{chub liver}]}$	$C_{[\text{parasite}]} / C_{[\text{chub stomach}]}$
	mean ± SD	mean ± SD	mean ± SD
Σ7 phthalates	22.8 ± 32.2 (***)	8.45 ± 7.73 (*)	7.71 ± 12.7 (*)
Σ16 PAHs	211 ± 187 (***)	86.2 ± 124 (**)	3.40 ± 1.50 (***)
Σ7 pyrethroids	1.51 ± 2.14	1.93 ± 2.99	1.94 ± 2.75
DEET	1.98 ± 0.99 (**)	2.05 ± 1.11 (*)	0.66 ± 0.91
Σ4 OCPs	6.60 ± 7.46 (***)	2.16 ± 1.00 (**)	0.43 ± 0.52 (*)
Σ7 PCBs	3.05 ± 1.86 (***)	1.98 ± 1.48 (*)	1.15 ± 0.77
Σ6 PBDEs	3.39 ± 2.44 (***)	0.76 ± 0.53	1.03 ± 0.86

<sup>a</sup>PAH = polycyclic aromatic hydrocarbons; DEET = *N,N*-diethyl-meta-toluamide; OCP = organochlorine pesticides; PCB = polychlorinated biphenyl; PBDE = polybrominated diphenyl ethers. The \* symbol indicates significant differences between the calculated ratio and the threshold value of 1 (\*)  $p < 0.05$ , (\*\*)  $p < 0.01$ , (\*\*\*)  $p < 0.001$ .

pyrethroids) were analyzed in four matrices: fish muscle (*n* = 118), liver (*n* = 26 and 93 for parent compounds and metabolites, respectively), stomach content (*n* = 35), and parasite tissues (*n* = 19 and 9 for parent compounds and metabolites, respectively). Due to a lack of biological material, chemical analyses were run on a reduced data set depending on the matrix. Between 0.1 and 0.5 g of freeze-dried and powdered fish matrix and 0.02 g of parasite tissues were used for quantitative analyses of parent compounds and their metabolites.

For all fish matrices, a freeze-dried aliquot of 0.1 g was analyzed to determine its lipid content (%). Following extraction with 2 × 5 mL of hexane:isopropanol (60:40, v/v), the residue was weighed to the nearest 0.1 mg for lipid determination as described in the work of Labadie et al.<sup>54</sup> The same method was applied for parasite tissue (0.05 g), and solvent volumes were adjusted proportionally to the aliquot.

**Statistical Analyses.** Descriptive statistics are reported as mean ± standard deviation (SD). Contaminant levels were not related to lipid content (all  $p > 0.05$ ). Consequently, the levels of contaminants (Σphthalates, ΣPAHs, Σpyrethroids, ΣOCPs, ΣPCBs, ΣPBDEs, Σphthalate metabolites, Σhydroxy-PAHs, and Σpyrethroid metabolites) are expressed in nanograms per gram of dry weight (see the work of Hebert and Keenleyside<sup>55</sup>). Data were analyzed using linear mixed models (LMMs) with the *lme4* package<sup>56</sup> in R v. 3.3.2 software.<sup>57</sup> The *lmerTest* package provided *p*-values via Satterthwaite's degrees of freedom method.<sup>58</sup> For each model, a backward elimination was used to progressively remove nonsignificant terms ( $p > 0.05$ ). Model assumptions were checked by examining residual plots.

**Objective 1.** We first aimed to test whether parasites preferentially accumulate organic pollutants. For this purpose a ratio of pollutant concentration in the parasite and in the host ( $r = C_{[\text{parasite}]} / C_{[\text{chub matrix}]}$ )<sup>59</sup> was calculated for each fish matrix (muscle, liver, and stomach content), with  $r > 1$  meaning a higher contamination of parasites compared to their host and the converse for  $r \leq 1$ . We tested whether the calculated ratio differed from the threshold value of 1, with *t*-test and nonparametric equivalent when the data did not meet normality assumptions. Spearman's tests were applied to evaluate the correlation between bioaccumulation ratios ( $r = C_{[\text{parasite}]} / C_{[\text{chub matrix}]}$ ) and chemical properties (hydrophobicity: log  $K_{OW}$ , metabolic rate: log  $k_M$ , and molecular weight), which were extracted from the EPA's EPI software suite (EPIWEB 4.1 package).<sup>60</sup>

**Objective 2.** Mixed models (LMMs) were applied to test whether parasite infection may reduce host contamination, with the log-transformed concentrations of either parent pollutants (*n* = 179, all fish matrices) or their metabolites (*n* = 93, liver) as dependent variables. We used parasite infection (two-level factor: infected or not), age,  $\delta^{15}\text{N}$ , lipid contents, and fish matrix (three-level factor: muscle, liver, and stomach content) as explanatory variables. Associations between variables were checked to avoid colinearity in the statistical models. Sampling site and fish identity were treated as random effects to account for variations between chub populations and to control for repeated measures of individuals, respectively. Pairwise comparisons of differences between infected and uninfected fish among the three matrices (muscle, liver, and stomach content) were tested using the *multcomp* package.<sup>61</sup>

**Objective 3.** We fitted LMMs to evaluate the link between biomarker response, parasite infection, and chemical exposure. OXY, d-ROMs, peroxidase, lysozyme, HSI, and K were dependent variables in our models. Infection status,  $\delta^{15}\text{N}$ , and age were entered as fixed effects. For model construction, we first evaluated the effect of the selected explanatory variables on the analyzed biomarkers, with sampling site as a random effect. Models were sequentially reduced by eliminating nonsignificant variables, and the effect of pollutant load (i.e., parent compounds in muscle, *n* = 118, and metabolites in liver, *n* = 93) was then individually tested on the final models.

## RESULTS

Data and descriptive statistics are summarized in Table S1. Of the 118 fish captured, 73 (61.9%) were infected by *Pomphorhynchus* sp., with a prevalence ranging from 50% to 90%, which did not differ across sampling sites ( $\chi^2 = 9.18$ , d.f. = 6,  $p = 0.16$ ). Infection probability did not vary with fish age (binomial GLMM:  $Z = -1.63$ ,  $p = 0.103$ ) but was positively influenced by body length ( $Z = -2.06$ ,  $p = 0.039$ ,  $\beta = 0.08 \pm 0.04$ ). Lipid contents (mean ± SD [range]) of fish matrices were the following: muscle  $3.01 \pm 1.72$  [0.56–8.98]%, liver  $6.19 \pm 3.99$  [1.59–14.5]%, and stomach content  $(8.76 \pm 4.77$  [3.35–19.8]%). There were no significant differences in lipid content between acanthocephalan parasites ( $4.71 \pm 1.37$  [2.75–5.81]%) and fish matrices (LMM: all  $p \geq 0.187$ ).

**Pollutant Distribution Inside the Host–Parasite System.** The relative accumulation of pollutants in parasites compared to their host depended on the family of pollutant analyzed (Table 1). For metabolizable pollutants, with the exception of pyrethroid pesticides, ratios ( $C_{[\text{parasite}]} /$

$C_{[\text{chub matrix}]}$ ) were significantly higher than 1, meaning that parasites accumulated higher levels of phthalates and PAHs than chub. For instance, *Pomphorhynchus* sp. accumulated ~2 to 700 times more  $\Sigma$ PAHs than the host, with levels of individual PAH congeners up to 5000 times higher in parasites than in fish muscle. In contrast, for persistent organic pollutants (POPs), parasite tissues exhibited similar or even lower levels of POPs compared to fish matrices (e.g., stomach contents; Table 1).

The pollutant burden in parasites significantly increased when compared with the levels in chub muscles for POPs ( $r_{\text{Pearson}} = 0.68$ ,  $t = 3.868$ ,  $p = 0.001$ , Figure S1) but not for metabolizable pollutants ( $p = 0.266$ ). In addition, POP ratios ( $C_{[\text{parasite}]} / C_{[\text{chub muscle}]}$ ) decreased with fish weight ( $r_{\text{Pearson}} = -0.46$ ,  $t = -2.123$ ,  $p = 0.049$  for OCPs; Figure S2) and length ( $r_{\text{Pearson}} = -0.50$ ,  $t = -2.402$ ,  $p = 0.028$ , and  $r_{\text{Pearson}} = -0.50$ ,  $t = -2.404$ ,  $p = 0.027$  for PCBs and PBDEs, respectively).

Bioaccumulation ratios ( $C_{[\text{parasite}]} / C_{[\text{chub muscle}]}$ ) increased with the metabolic transformation rate of the molecule ( $\log k_M$ ,  $r_{\text{Spearman}} = 0.34$ ,  $p = 0.019$ ; Figure 2) but were not correlated to its molecular weight ( $p = 0.506$ ) nor its hydrophobicity ( $\log K_{OW}$ ,  $p = 0.339$ ).

#### Effect of Parasites on Their Hosts' Pollutant Burdens.

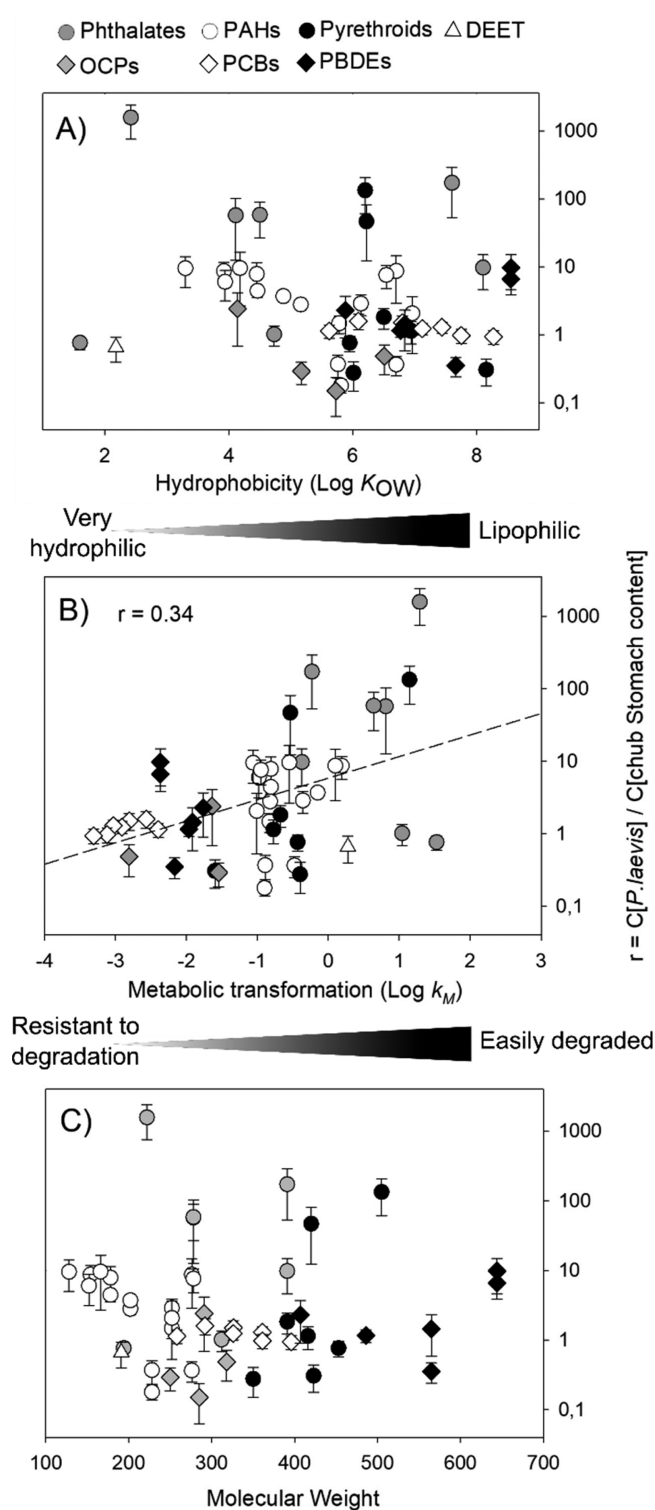
The pollutant loads of parent compounds and metabolites in infected and uninfected chub are presented in Figure 3. No significant differences were detected whatever the fish matrix considered (Figure 3, Tables S2 and S3). In addition, metabolite levels in the liver did not differ significantly between infected and uninfected fish (all  $p \geq 0.182$ ; Table S2) and were not statistically different from the levels in parasites (Figure 3; all  $F_{(2,99)} \geq 1.69$ ,  $p \geq 0.188$ ), except for pyrethroid metabolites. However, it should be noted that out of the 19 metabolites investigated, only 6 were detected in more than half of the parasite samples and all pyrethroid metabolites were below the limit of quantification (<LOQ).

High interindividual variability in parent pollutant load was observed but was not correlated to age (range: 1–9 years, all  $p \geq 0.222$ ; Supporting Information Table S2),  $\delta^{15}\text{N}$  (5.92–12.9‰, all  $p \geq 0.071$ ), or lipid contents (0.56–19.8%, all  $p \geq 0.085$ ). The level of metabolites in chub liver increased significantly with age (phthalate, LMM:  $F_{(1,85)} = 16.9$ ,  $P < 0.001$ ,  $\beta = -0.11 \pm 0.03$ ; Table S2, and PAH,  $F_{(1,85)} = 4.65$ ,  $p = 0.034$ ,  $\beta = -0.12 \pm 0.06$ ).

**Effect of Pollutant Load and Parasite Infection on Biomarkers.** Detailed information on variations in biomarker response among sampling sites and according to individual traits are provided in the Supporting Information (Tables S4, S5, and S6).

**Molecular/Cellular Levels.** Oxidative damage was significantly lower in infected chub compared to uninfected fish (d-ROMs;  $F_{(1,78.67)} = 6.01$ ,  $p = 0.016$ ,  $\beta = 0.21 \pm 0.08$ ; Figure 4) but was not related to the pollutant load (d-ROMs, all  $p \geq 0.171$ ; Supporting Information Table S4). Total plasma antioxidant capacity was unrelated to the presence of parasites (OXY; LMM:  $F_{(1,92.20)} = 0.04$ ,  $p = 0.833$ ) but decreased with the levels of phthalate metabolites (OXY,  $F_{(1,73.89)} = 6.96$ ,  $p = 0.010$ ,  $\beta = -46.8 \pm 17.7$ ; Figure S3) and the sum of all metabolites ( $F_{(1,73.67)} = 4.38$ ,  $p = 0.039$ ,  $\beta = -41.0 \pm 19.6$ ) in the liver.

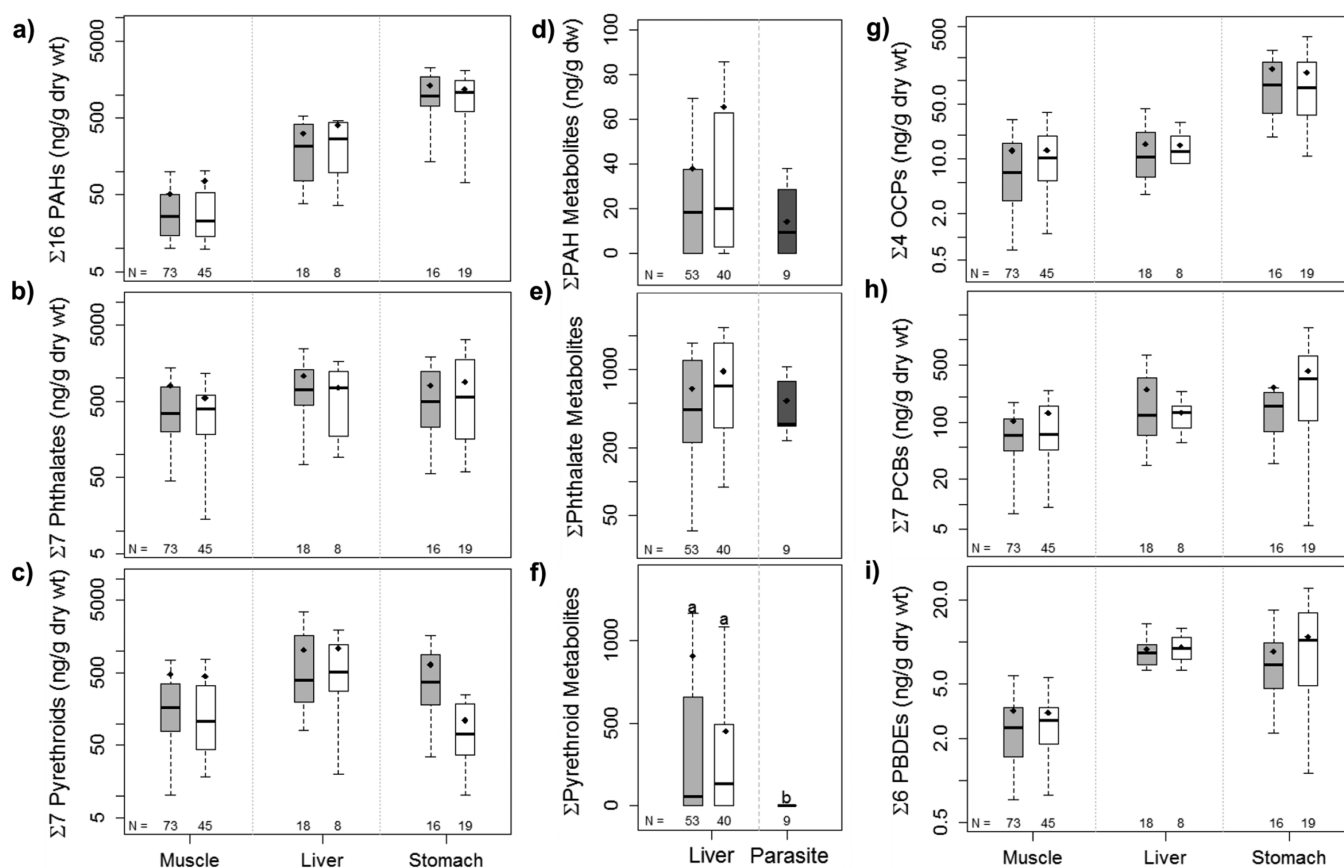
Neither peroxidase (LMM:  $F_{(1,69.57)} = 0.32$ ,  $p = 0.574$ ) nor lysozyme activity ( $F_{(1,62.88)} = 0.18$ ,  $p = 0.674$ ) were affected by the presence of parasites. In addition, peroxidase activity decreased with levels of PAH metabolites ( $F_{(1,60)} = 6.00$ ,  $p =$



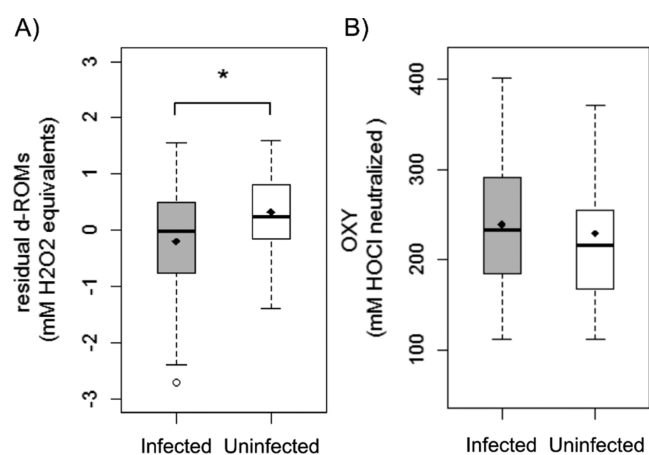
**Figure 2.** Bioaccumulation ratio ( $r$ ; mean  $\pm$  SD) of 48 organic pollutants ( $\text{ng g}^{-1}$  of dry wt) in *Pomphorhynchus* sp. relative to fish stomach content in relation to (A) their hydrophobicity ( $\log K_{OW}$ ), (B) metabolic biotransformation ( $\log k_M$ ), and (C) molecular weight [PAHs = polycyclic aromatic hydrocarbons; PBDE = polybrominated diphenyl ether; DEET = *N,N*-diethyl-meta-; PCB = polychlorinated biphenyl; OCP = organochlorine pesticides].

$0.017$ ,  $\beta = -0.30 \pm 0.12$ ; Figure S3). In contrast, lysozyme activity was not affected by the pollutant load (Table S5).

**Organ/Organismal Levels.** Neither body condition nor hepatosomatic index were related to infection status (LMM:



**Figure 3.** Boxplot of the pollutant load ( $\text{ng g}^{-1}$  dry wt; [a–c] metabolizable and [d–f] their byproducts, [g–i] persistent) in the muscle, liver, and stomach content of infected (gray) and uninfected (white) chub. Numbers below boxplots refer to the sample size.



**Figure 4.** Effect of infection status (parasitized or not) on (A) oxidative damage (d-ROMs) and (B) total plasmatic antioxidant capacity (OXY). Residuals from the final model (see Table S4), excluding the infection status, were used to correct for variation caused by confounding factors (age and  $\delta^{15}\text{N}$ ). Filled diamonds represent the arithmetic mean, and the asterisk indicates a significant difference ( $p < 0.05$ ).

## DISCUSSION

The present study investigated the potential benefits of *Pomphorhynchus* sp. on its final host *S. cephalus* exposed to organic pollutants, especially on biomarker responses and on the pollutant load in hosts, given the expected accumulation of metabolizable and water-soluble molecules by parasites.

As expected, intestinal parasites had higher accumulation capacities compared to their host for most of the pollutants investigated, which is consistent with previous studies on trace metals and PCBs.<sup>15,16,26,62</sup> Because of their lack of digestive tract and basic synthesis pathways, acanthocephalan parasites take up substances (e.g., lipids) directly from the host intestine.<sup>63</sup> It is thus likely that pollutants in acanthocephalan tissues result from an indirect transfer from chub to parasite. Alternatively, the pollutant load in *Pomphorhynchus* sp. might result from previous exposure in the intermediate host (i.e., in gammarids), yet chemicals were found to be predominantly accumulated by acanthocephalans inside the definitive host.<sup>64</sup> Pollutant accumulation patterns in our host-parasite system were highly variable but were unrelated to biological factors, such as  $\delta^{15}\text{N}$  and lipid content. However, the accumulation of persistent organic compounds (POP) in parasites relative to their host ( $C_{\text{parasite}}/C_{\text{chub muscle}}$ ) decreased with increasing fish weight or length, likely because of their high lipophilicity ( $\log K_{\text{OW}} > 6$ ), their resistance to degradation and their bioaccumulation potential in fish during growth.<sup>65</sup> Interestingly, metabolizable pollutants (PAHs and phthalates) had the highest accumulation ratios. One may predict that acanthocephalan parasites are less able to degrade and excrete organic

$F_{(1,109)} = 0.21$ ,  $p = 0.645$ ;  $F_{(1,107.97)} = 0.02$ ,  $p = 0.884$  respectively) or pollutant load (all  $p \geq 0.077$ ), except for a negative correlation between OCP levels and hepatosomatic index ( $F_{(1,105.66)} = 6.13$ ,  $p = 0.015$ ,  $\beta = -0.47 \pm 0.19$ , Figure S3).



pollutants than their final host, because of weaker metabolic efficiency measured in the major tissues involved in the digestive system of invertebrates compared to the liver of vertebrates.<sup>23,66</sup> Specifically, our study reveals that molecules with  $k_M > -1$  accumulated preferentially in parasites, suggesting a key role of metabolic processes in the differential distribution of pollutants within the host-parasite system. Still, the detection of byproducts in *Pomphorhynchus* sp. highlights either that parasites are able to metabolize certain chemicals<sup>67,68</sup> or that they incorporate metabolites readily biotransformed inside host tissues. Since our study is cross-sectional, we could not exclude the “selection hypothesis” stating that infected fish bearing a high pollutant load are progressively eliminated from the population. Additionally, sex was not recorded in this study, but gender-specific physiological mechanisms (i.e., metabolic and elimination pathways) might affect pollutant bioaccumulation.<sup>69</sup>

Contrary to our expectations and despite the preferential accumulation of pollutants in acanthocephalans compared to their host, contamination levels in parasitized chub were not significantly different from unparasitized ones. These results are in line with a recent study pointing out that dogs with or without heartworm parasites (*Dirofilaria immitis*) exhibited similar serum levels of PAHs and PCBs.<sup>18</sup> However, unlike acanthocephalans, heartworm parasites feed on their host tissues or fluids, which likely induces a greater uptake of pollutants from the host compared to intestinal parasites. Additionally, extrapolation of ecotoxicological and ecological results from domestic to wild animals should be conducted carefully. Still, past findings have evidenced lower levels of trace metals<sup>12,14</sup> and persistent pollutants<sup>68,70</sup> in infected hosts compared to unparasitized ones. A potential explanation is the weak parasitic load encountered in our study (median [range] of parasite biomass per fish, 14.8 [0.99–500] mg of dry wt), probably lessening the detoxification process of infected fish. Moreover, environmental conditions, especially pollution, may positively or negatively influence host–parasite interactions,<sup>71</sup> by increasing parasite abundance and diversity in polluted waters<sup>72</sup> or by compromising the survival of intermediate hosts (e.g., gammarids)<sup>73</sup> and the life cycle of parasites. In the present study, the prevalence of *Pomphorhynchus* sp. did not differ among sampling sites despite the high variation of organic pollution in water and sediments (e.g.,  $\sum$ PCBs: 2.04–57.5 ng g<sup>−1</sup> dry wt, data not shown), but the abundance of infected gammarids was not monitored.

One of the key findings was that the oxidative status of chub was related to parasitic infection in chemically altered environments. More specifically, infected chub exhibited significantly lower oxidative damage compared to uninfected ones, but did not differ in their antioxidant capacity. Interestingly, the pollutant load had no significant effect on oxidative damage, suggesting that the accumulation of chemicals in parasites could not solely explain the positive effect of intestinal parasites on chub. Our results support the hypothesis that acanthocephalans are conditionally helpful parasites for chub under polluted conditions<sup>4</sup> as enhanced oxidative damage and potentially oxidative stress, in unparasitized chub could drive fitness costs for the host (see the work of Costantini<sup>74</sup>). In fact, even stronger impacts on the oxidative status may be expected, since phthalate metabolites were associated with lower antioxidant capacity in chub, which is further supported by Qu et al.<sup>34</sup> Recent findings also evidenced that parasites were beneficial to crustacean

intermediate hosts exposed to pollution, by increasing antitoxic defenses,<sup>30,75</sup> thus favoring their own survival and transmission to the final host. Possible explanations for our results include the sequestration of chemicals in parasites, which might affect the internal distribution and levels of contaminant in fish.<sup>11,16</sup> Consequently, intestinal parasites may mitigate the production of highly reactive molecules generated from parent pollutants, thereby lowering toxicity.<sup>5</sup> This explanation is however not fully supported by our results, since infected and uninfected chub did not differ in their pollutant load. On the other hand, individual quality could be a potential confounding factor. Chub that feed more frequently are indeed more likely to get infected and should exhibit a better oxidative status, thus being “healthier” hosts.<sup>76</sup> Only an experimental approach in which exposure levels and/or parasitism are controlled can help to disentangle such confounding factors.

At higher biological levels, there was no association between parasite infection and the health status of fish, whereas immune activity (peroxidase) and condition indices (HSI) were found to decrease with increasing pollutant load. While Dezfouli et al.<sup>8</sup> evidenced local damage and inflammatory reactions in fish due to acanthocephalan infections, our results did not support such negative effects of parasites on body condition and immune activities. The most likely explanation may be related to the low parasite load of *Pomphorhynchus* sp. in chub and/or to the low immune activation by this parasite. Indeed, the biomass of intestinal parasites inside chub intestine was probably too low to trigger clear differential responses between infected chub and parasite-free individuals. Interestingly, a recent finding pointed out a quadratic relation between body condition and parasite abundance or biomass,<sup>41</sup> with benefits at intermediate parasite loads.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.0c00177>.

Figure S1, patterns of organic pollutant bioaccumulation in the host–parasite system; Figure S2, relationships between organic pollutant bioaccumulation and chub characteristics; Figure S3, biomarker responses to chub characteristics and chemical exposure; Table S1, morphological and biological data; Tables S2, S3, mixed model outputs testing the effect of parasite infection on the host's pollutant load; Table S4, morphological and biological data at each sampling site; Tables S4, S5, mixed model outputs testing the effects of parasite infection and chemical exposure on biomarker responses (PDF)

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## Notes

The authors declare no competing financial interest.

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