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Host-parasites transfer of micropollutants and eco-physiological consequences on a freshwater fish: case study of chub-acanthocephalan model

Noëlie Molbert

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Noëlie Molbert. Host-parasites transfer of micropollutants and eco-physiological consequences on a freshwater fish: case study of chub-acanthocephalan model. Ecotoxicology. Sorbonne Université, 2020. English. NNT: 2020SORUS102 . tel-03214879

HAL Id: tel-03214879

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Submitted on 3 May 2021

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Sorbonne Université

Doctoral school GRNE 398

*UMR 7619 Milieux Environnementaux, Transferts et Interactions dans les hydrosystèmes et
les Sols (METIS) / Département Biogéochimie*

Host-parasites transfer of micropollutants and eco- physiological consequences on a freshwater fish:

Case study of chub-acanthocephalan model

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Speciality: Ecotoxicologie

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Sorbonne Université

Ecole doctorale GRNE 398

*UMR 7619 Milieux Environnementaux, Transferts et Interactions dans les hydrosystèmes et
les Sols (METIS) / Département Biogéochimie*

Transfert hôte-parasites de micropolluants et conséquences éco-physiologiques sur un poisson d'eau douce : *Cas du modèle chevesne-acanthocéphale*

Par **Noëlie Molbert**

Thèse de doctorat d'Ecotoxicologie

Dirigée par Aurélie Goutte et Jean-Marie Mouchel

Présentée et soutenue publiquement le 17 décembre 2020

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Preface

1. Abstract:

Exposure to complex mixtures of environmental contaminants may have severe consequences in free-living animals through the disruption of physiological mechanisms, reduced reproductive outputs and survival, thereby leading to population collapse. Contaminant analyses in fish have been used as a routine approach in studies of aquatic pollution. Located at the upper levels of the food webs, fish are indeed particularly exposed to chronic contamination. Under natural conditions, organisms are also exposed to a vast array of stressors, including parasites. Both chemical exposure and parasite infection have been well studied and documented, but have in many cases been investigated independently from one another. However, it is crucial to simultaneously assess their combined effect on wild organisms given that parasites may interfere with the fate of environmental contaminants within their host through their bioaccumulation capacity. This thesis focuses on six families of organic contaminants, and some of their metabolites, including persistent pollutants still reported at sublethal levels in freshwater fish despite their ban from use and production, (Polychlorinated biphenyl: PCBs, organochlorine pesticides: OCPs and Polybrominated diphenyl ethers: PBDEs) as well as substances broadly use in industrial processes, agriculture and in residential areas (Polycyclic aromatic hydrocarbons: PAHs, phthalates, insecticides).

Based on a field study, completed by an experimental approach, I investigated the fate and consequences of these organic contaminants in a host-parasite system composed of a freshwater fish, the European chub, *Squalius cephalus*, and its intestinal parasite, *Pomphorhynchus* sp. from the Marne River, France. Specifically, I investigated whether intestinal parasites were able to accumulate toxicants and how their presence affected the stress response of their definitive host exposed to environmental contaminants. This was examined at different biological levels with the use of general biomarkers providing information on their immune and oxidative physiological state (lysozyme, peroxidase, antioxidants, oxidative damage), the diversity and composition of gut bacterial communities (microbiota), their body condition (Fulton's index and hepatosomatic index) and life expectancy (telomere length). This PhD work underlines the toxicity of metabolites of organic contaminants, which were associated with increased oxidative damage, reduced antioxidant defenses and shorter telomeres. Importantly, we demonstrated that intestinal worms were able to accumulate organic contaminants, detoxify their hosts and that their effects on the oxidative balance shifted from negative to positive as chemical exposure increased. Evidence from both our field and laboratory studies therefore suggests that natural infection by intestinal worms can modulate the host's stress response to toxicants through physiological changes, which might benefit the host under polluted condition.

Keywords: Aquatic ecotoxicology, Organic micropollutants, Metabolites, Biotic interaction, Ecophysiology, European chub

Résumé:

L'exposition à des mélanges complexes de substances chimiques dans l'environnement peut avoir de graves conséquences pour les animaux sauvages, à travers la modification de mécanismes physiologiques, la réduction des taux de reproduction et de survie, entraînant ainsi l'effondrement des populations. Les analyses de contaminants chez les poissons ont été utilisées comme approche de routine dans les études portant sur la pollution aquatique. Situés dans les maillons supérieurs des chaînes alimentaires, les poissons sont en effet particulièrement exposés à la contamination chronique. En milieux naturels, ces organismes sont également affectés par de nombreux facteurs de stress, y compris le parasitisme. L'exposition chimique et l'infection parasitaire ont toutes deux été bien étudiées et documentées, mais dans de nombreux cas, elles ont été étudiées indépendamment l'une de l'autre. Cependant, il est crucial d'évaluer simultanément leur effet combiné sur les organismes sauvages étant donné que les parasites peuvent interférer sur le devenir des polluants environnementaux chez leur hôte grâce à leur capacité de bioaccumulation. Cette thèse se concentre sur six familles de contaminants organiques, et certains de leurs métabolites, incluant des polluants persistants encore détectés à des niveaux sub-létaux dans les poissons d'eau douce malgré leur interdiction d'utilisation et de production (Polychlorinated biphenyl : PCB, pesticides organochlorés : OCP et éthers diphényles polybromés : PBDE), ainsi que des substances largement utilisées dans les processus industriels, l'agriculture et les ménages (Hydrocarbures aromatiques polycycliques : HAP, phtalates, insecticides).

Sur la base d'une étude de terrain, complétée par une approche expérimentale, j'ai étudié le devenir et les conséquences de ces contaminants organiques dans un système hôte-parasite composé d'un poisson d'eau douce, le chevesne, *Squalius cephalus*, et de son parasite intestinal, *Pomphorhynchus* sp. issus de la Marne, en France. Plus précisément, j'ai cherché à savoir si ces vers intestinaux étaient capables d'accumuler des substances toxiques et comment leur présence affectait la réponse au stress de leur hôte exposé aux contaminants environnementaux. Cette étude a été réalisée à différents niveaux biologiques à l'aide de biomarqueurs généraux fournissant des informations sur leur état physiologique (lysozyme, peroxydase, antioxydants, dommages oxydatifs), la diversité et la composition des communautés bactériennes intestinales (microbiote), leur état corporel (indice de Fulton et indice hépatosomatique) et leur espérance de vie (longueur des télomères). Ce travail de doctorat souligne la toxicité des métabolites de contaminants organiques, qui ont été associés à une augmentation des dommages oxydatifs, à une réduction des défenses antioxydantes et à des télomères plus courts. Il est important de noter que nous avons démontré que ces vers intestinaux étaient capables d'accumuler des contaminants organiques, de détoxifier leurs hôtes et que leurs effets sur l'équilibre oxydatif transitaient de négatifs à positifs à mesure que l'exposition aux contaminants organiques augmentait. Les résultats de nos études sur le terrain et en laboratoire suggèrent donc que cette infestation naturelle par certains vers intestinaux peut moduler la réponse au stress de l'hôte, exposé à des substances toxiques, par des changements physiologiques, ce qui pourrait être bénéfique à l'hôte dans des environnements pollués.

Mots clés : Écotoxicologie aquatique, Micropolluants organiques, Métabolites, Interaction biotique, Ecophysiologie, Chevesne

2. Acknowledgements/ Remerciements

Incontestablement cette partie aura été la plus compliquée à écrire. Je ne compte plus le temps passé à fixer cette page en pensant à la façon de débiter cette longue série de remerciements ! Il faut dire que cette thèse aura été le théâtre de nombreuses rencontres, en partie dues au caractère pluridisciplinaire de ce sujet. Je garde de chacune d'entre elles de précieux souvenirs. A leur manière, les différentes personnes citées auront toutes contribué au succès de ces trois dernières années, en me guidant et en me soutenant tout au long de cette magnifique aventure.

En tout premier lieu, je tiens à adresser mes plus sincères remerciements à mes directeurs de thèse : **Aurélie Goutte** et **Jean-Marie Mouchel**. Il est évident que sans vous je n'en serais pas là aujourd'hui ! Je suis très fière d'avoir réalisée cette thèse à vos côtés. Merci de m'avoir poussé à persévérer et à me maintenir motivée pour obtenir une bourse doctorale. Je n'aurais pu rêver d'une meilleure équipe encadrante, avec deux chercheurs toujours à l'écoute et qui fourmillent d'idées (peut-être trop parfois). Merci de m'avoir laissé les rênes et d'avoir eu confiance en moi tout au long de cette thèse. Il faut aussi dire le nombre de fois où j'ai débarqué devant vos bureaux avec des papiers à signer, des articles à relire où simplement des questions à poser... Vous avez toujours répondu présent et pour ça mille mercis. Merci de m'avoir toujours tirée vers le haut et merci pour cet épanouissement scientifique tout au long de ces trois années. Merci aussi à Aurélie de m'avoir permis de barboter dans l'eau en plein mois de janvier sous la neige à -5°C et d'avoir pu pêcher dans les coins les plus vaseux de notre planète :) ... expérience inoubliable ! Je suis extrêmement reconnaissante d'avoir pu participer à des projets annexes et effectuer mes premiers pas dans l'enseignement.

Merci aux membres du jury **Julien Gasparini**, **Stéphane Bétouille**, **Hélène Fenet**, **Séverine Jean**, **Paco Bustamante**, **Thierry Rigaud** d'avoir accepté d'évaluer ce travail de thèse et pour leurs retours très constructifs ! Merci pour ces échanges passionnants qui ouvrent aujourd'hui sur tout un tas de pistes à explorer. J'espère que cette soutenance aura été tout aussi plaisante pour vous qu'elle l'a été pour moi.

Un grand merci aussi aux membres du comité de thèse **Julien Gasparini**, **Lisa Jacquin**, **Fabienne Petit**, **Jérémy Lebrun** et **Vincent Médoc**. J'ai énormément appris à vos côtés et nos échanges, très enrichissants, ont indéniablement influencé le déroulé et la réussite de cette

thèse. Merci d'avoir partagé vos expériences respectives et d'avoir toujours été si bienveillants. Je suis toujours ressortie plus motivée que jamais de nos entretiens.

Je tiens également à remercier toutes les personnes avec lesquelles j'ai collaboré et tous les laboratoires qui m'ont vu passer aux cours de ces trois années. Merci à **Frédéric Angelier** et **Cécile Ribout** du CEBC (Centre d'études Biologiques de Chizé), je suis maintenant incollable sur les techniques d'extraction et dosage d'ADN. Merci également aux doctorants, stagiaires et personnels du CEBC d'avoir rendu mes différents séjours au centre aussi plaisants. Un grand merci aussi à **Béatrix Decencière**, **Alexis Millot** et **Simon Agostini** au CEREEP (Centre de Recherche en écologie expérimentale et prédictive). Sans vous l'expérience avec les chevesnes aurait été difficilement réalisable. Merci pour votre aide dans le montage du dossier d'éthique, pour avoir si bien veillé et pris soin de mes poissons en mon absence et pour tous les moments partagés qui ont contribué à rendre cette expérience inoubliable. J'ai énormément appris à vos côtés (manipulation des poissons, pose des PIT-tags, entretien des aquariums, nourrissage, etc...) et bien que les journées aient été longues et éprouvantes, cela a toujours été pour moi un réel plaisir de revenir, jour après jour. Je remercie aussi **Clotilde Biard**, **Sandrine Meylan** et **Mathieu Leroux-Coyau** à l'iEES (Institut d'écologie et des sciences de l'environnement) pour votre expertise et vos retours. Merci Mathieu d'avoir rendu tous ces dosages un peu plus fun ! Je me souviendrai toujours du suspense insoutenable lors de la lecture des plaques :). Merci aussi à **Fabienne Petit**, **Thierry Berthe** et **Yannick Colin** du M2C. Cette plongée dans le monde de la microbiologie fut passionnante. Je vous remercie pour votre accueil, votre gentillesse et votre aide précieuse. Merci à **Mercedes Mendez** et **Magloire Mandeng-Yogo** de l'IRD pour les analyses isotopiques. Enfin merci à **Raphaël Santos** pour tes précieux conseils et ton énergie débordante.

Je souhaite également remercier l'ensemble de l'équipe EPHE pour tout ce qu'ils m'ont apporté pendant cette thèse. Merci à **Sylvie Derenne**, **Hélène Blanchoux**, **Elodie Guigon**, **Fabrice Alliot**, **Thomas Thiebault**, **Catherine Bourges** et **Annie Desportes**. Merci pour votre soutien et les innombrables conseils. J'ai eu la chance d'avoir une équipe très soudée et dynamique. J'ai réellement pris plaisir à travailler à vos côtés. Un grand merci à Annie et Catherine d'avoir rythmé mes journées au labo, tantôt dans le froid (avec le bonnet sur la tête et écharpe autour du cou) et tantôt sous la chaleur écrasante des mois d'été. Merci pour votre aide lorsque je croulais sous les analyses. Bonne retraite à toutes les deux !

Je souhaite également remercier un certain nombre de chercheurs que j'ai eu l'occasion de contacter ou rencontrer au cours de divers colloques et workshops : **Vlatka Filipović Marijić, Omar Amin, Yen Le, Bernd Sures** et **Thierry Rigaud**. Vous m'avez apporté une aide précieuse pour l'expérience avec les acanthocéphales et vous m'avez transmis votre passion pour les parasites. En tant que novice, je suis sortie grandie de nos rencontres et discussions. Je n'oublierai jamais le séjour en Slovaquie et le Tatratea !

Je remercie également les différents organismes qui ont financé cette thèse, dont **l'Institut de la Transition Environnementale** de Sorbonne Université (SU-ITE). L'ITE, qui a financé cette thèse et les formations, a su déceler l'énorme potentiel de ce sujet. Je ne les remercierai jamais assez d'avoir permis la concrétisation de cette thèse. Merci également à **l'agence de l'eau Seine-Normandie** et le **PIREN-Seine**. Merci aussi à **l'EPHE** et à **METIS** pour les financements sur la partie expérimentale.

Merci à **Valérie Girard, Jean-Robert Gowe, Nadine Bost, Bénédicte Sabatier, Alban Bakhti** et **Francis Chabrier** pour avoir rendu toutes ces procédures administratives moins douloureuses : missions, colloques, devis, contrat et j'en passe ! Merci pour votre efficacité et votre sympathie.

A mes collègues **Danièle, Paul** (alias le poulpe ;)), **Ludovic, Sylvain, Marc, Marie, Thomas, Audrey, Aurélien, Valérie, Vincent** et **Pierre R** qui m'ont perçue comme un « Terminator » tout au long de ces trois années et qui n'ont pas manqué de se moquer d'une de mes compétences les plus incroyables, celle de réanimer les poissons (Danièle je te transmets le flambeau). Bien que noyée dans la masse des hydrologues/hydrogéologues et modélisateurs, le couloir du 4^{ième} restera à jamais gravé dans ma mémoire et j'espère avoir à nouveau l'occasion de travailler dans un tel environnement de convivialité et de partage. Une pensée spéciale à tous les occupants actuels et passés du bureau 410 avec qui j'ai refait le monde et sans qui rien n'aurait vraiment pas été pareil. Je pense bien évidemment à **Raphaël** (et **Mae**, qui sont aujourd'hui deux amis sur lesquels je peux compter dans les bons comme les mauvais moments), **Ana, Ardalan, Ningxin** (à qui je dois ma première dégustation de méduse, mon premier Roland Garros et qui m'a gâtée et nourrie pendant toutes ces années), **Mounir, Pierre** (le génie de R et mon fidèle disciple, je dirais que notre relation s'apparente à celle du Dr Frankenstein et sa chose) et **Thibault** (à qui je dois l'utilisation de nouvelles expressions). Il est difficile d'expliquer en quelques mots tout ce qu'ils ont pu m'apporter : des délires, des

après-midis « école buissonnière » au centre commercial de La Défense, des restaurants et pique-niques, des pauses café avec un bon petit pain au chocolat, etc... Merci aussi à **Mohamed** (« Momo ») d'avoir toujours répondu présent lorsque j'avais une question (c'est-à-dire presque tous les jours). Merci d'avoir apporté ton grain de folie/philosophie au couloir et avoir contribué à cette atmosphère bon enfant. **Pedro** et Momo, vous avez rendu les week-ends passés au bureau plus supportables avec nos mythiques Kebab. Merci à toi aussi Salma et ta force tranquille. Je te souhaite le meilleur aux States ! **Flore**, tu as été une des révélations de ce couloir. Arrivée de Québec et ancrée à METIS suite à l'épidémie de COVID, tu as été une véritable bouffée de bonne humeur. Merci de m'avoir accompagnée dans mes crises de boulimie avec nos fameux goûters. A **Diane** aussi, qui fait partie du noyau dur de toute cette bande. Tu as été (et tu le resteras) ma confidente et amie tout au long de cette thèse. Nos soirées, le match France-Turquie et notre plongée 4**** à l'époque de l'empereur Hadrien resteront à jamais gravés dans ma mémoire. Tu as été ma complice de labo et tu as rendu certains moments beaucoup plus supportables. La thèse est une de ces aventures qui permet de révéler certaines personnes et de créer des liens d'amitié très forts. Diane, Rafa et Mae, vous êtes de ces personnes ! Enfin merci à tous les autres doctorants/ATER/pots-doc : **Alexandre, Xingcheng, Flore R** (mon binôme de conseil d'unité au cours de ces trois années), **Aïda, Pierre V, Etienne, Kawtar, Elijah, Axel, Karim, Loïc** et **Nouradine**. Merci à vous tous.

Merci à **Marwa Khabou**, la meilleure stagiaire qu'un doctorant puisse avoir. Merci à toi d'avoir participé à la douloureuse épreuve de lyophiliser, broyer, peser, analyser tout un tas de matrices biologiques à mes côtés. Je te souhaite tout le meilleur à l'école 42 !

Merci à mes amis(es) et colocataires **Amélie, Emmanuelle, Lisa** et **Mathieu**. Vous avez été mes cobayes avant chaque comité de thèse et je vous remercie d'avoir été là pour me changer les idées lorsque j'en avais besoin. Merci à Amélie pour m'avoir « initiée » à la pole dance ;) . Je garde de merveilleux souvenirs de notre vie à Alfortville.

A ma famille surtout, dont **mes parents** qui m'ont apporté un soutien sans faille. Merci pour vos encouragements, votre implication et votre bienveillance, surtout dans les moments de doute ! A **ma mère** pour la relecture de mes documents (reviewer en or) et qui a soigneusement recopié l'intitulé de ma thèse et de mon parcours pour être en mesure d'expliquer au reste de la famille ce que la « petite dernière » fabriquait avec les poissons. Merci à **mamie** d'avoir été mon minuteur humain en me demandant chaque mois : « Mais quand est-ce que ça fini ton

machin ? ». Chaque appel me permettait ainsi d'apprécier le temps restant avant la fin de ma thèse. Enfin, merci à ma sœur chérie **Elise**, dont je tairai le surnom, qui est à la fois ma meilleure amie, mon mentor et modèle et ma plus fervente supportrice.

3. Scientific Production

3.1 List of Publications

These publications are attached at the end of the manuscript.

- [4] **Molbert N**, Millot A, Decencière B, Collin Y, Leroux-Coyau M, Alliot F, Berthe T, Petit F, Goutte A. (*Submitted*) Parasitism reduces oxidative stress and alters gut microbiota of fish host experimentally exposed to PAHs. ¹
- [3] **Molbert N**, Angelier F, Alliot F, Ribout C, Goutte A. Fish from urban rivers and with high pollutant levels have shorter telomeres. *Biol Letters* **2021**, 20200819. (doi: 10.1098/rsbl.2020.0819) (Impact factor: 3.32).
- [2] **Molbert N**, Alliot F, Leroux-Coyau M, Médoc V, Biard C, Meylan S, Jacquin L, Santos R, Goutte A. Potential benefits of acanthocephalan parasites for chub hosts in polluted environments. *Environ. Sci. Technol.* **2020**, 54, 5540–5549. (doi:10.1021/acs.est.0c00177). (Impact factor: 7.14).
- [1] **Molbert N**, Alliot F, Santos R, Chevreuil M, Mouchel JM, Goutte A. Multi-residue methods for the determination of organic micropollutants and their metabolites in fish tissues. *Environ. Toxicol. Chem.* **2019**, 38, 1866–1878. (doi:10.1002/etc.4500). (Impact factor: 3.18).

Publications not related to the thesis:

- [2] Colin Y, Berthe T, **Molbert N**, Guigon E, Vivant AL, Alliot F, Collin S, Goutte A, Petit F. Urbanization constrains skin bacterial phylogenetic diversity in wild fish populations and correlates with the proliferation of aeromonads. *Microbial Ecology* **2021** (doi: 10.1007/s00248-020-01650-2) (Impact factor: 3.6).
- [1] Goutte A, **Molbert N**, Guérin S, Richoux R, Rocher V. Monitoring freshwater fish communities in large rivers using environmental DNA (eDNA) and a long-term electrofishing survey (1990-2018). *J. Fish Biol.* **2020**, 1–9 (doi:10.1111/jfb.14383). (Impact factor: 2.03).

3.2 Conference – Workshop participation (Oral)

- [5] Goutte A, Labadie P, **Molbert N**, *et al.* Transfert trophique de contaminants organiques dans le bassin de la Seine «Colloque de la Zone atelier - Seine»; Paris (reporté en 2021).

¹this manuscript is going to be divided into 2 parts (*i.e.*, gut microbiota and ecophysiological results) following returns from the editors

- [4] **Molbert** N, Mouchel JM, Goutte A. Transfert hôte-parasites de micropolluants chez un poisson sentinelle de l'anthropo-hydrosystème Seine. « Journée Sorbonne Université-Institut de la transition environnementale (SU-ITE) ». Paris, France (11 Janvier 2019).
- [3] **Molbert** N, Alliot F, Médoc V, Mouchel JM, Goutte A. Insights from chub-acanthocephalan system in polluted environments: towards a host decontamination? In: Update on selected topics in acanthocephalan parasites research. *Helminthologia* **2018**, 55, 350–562. (doi:10.2478/helm-2018-0023). (Impact factor: 0.73). « 9th Acanthocephalan Workshop »; Stara Lesna, Slovaquie (9-13 Septembre 2018).
- [2] Goutte A, Alliot F, **Molbert** N, Chevreuil M. Métabolisation des micropolluants organiques: Imprégnation et dommages potentiels chez les chevesnes (*Squalius cephalus*). Société Française d'Ecotoxicologie Fondamentale et Appliquée, Montpellier, France (27-28 juin 2018)
- [1] **Molbert** N, Alliot F, Santos R, Chevreuil M, Goutte A. Levels of several organic micropollutants and their metabolites in the European chub from the Marne hydrographic network. «13th Ecology and Behavior meeting »; Chizé (CEBC), France (19-23 Juin 2017).

3.3 Conference – Workshop participation (Poster)

- [4] **Molbert** N, Fabrice A, Leroux-Coyau M, Médoc V, Biard C, Meylan S, Jacquin L, Santos R, Goutte A. Are acanthocephalan parasites conditionally helpful for chub in polluted environments ? « 30th Annual Meeting of the Society of Environmental Toxicology And Chemistry (SETAC) » ; Dublin, Irlande (4-7 Mai 2020).
- [3] **Molbert** N, Guérin S, Rocher V, Goutte A. Comparaison entre pêche d'inventaire et AND environnemental pour appréhender la diversité piscicole sur la Seine et de la Marne en région parisienne. « 23e édition des Journées Informations Eaux » ; Poitiers, France (9-11 Octobre 2018).
- [2] **Molbert** N, Chevreuil M, Alliot F, Santos R, Mouchel JM, Goutte A. Impregnation factors of freshwater fish by organic micropollutants in the Marne hydrographic network. « 28th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) »; Rome, Italy (14-17 Mai 2018).
- [1] **Molbert** N, Goutte A, Alliot F, Mouchel JM. Impregnation factors of freshwater fish by organic micropollutants in the Seine river catchment. « International Long Term

Ecological Research Network & LTER-France (Zones Ateliers Network & Critical Zone Observatories) »; Nantes, France (2-4 Octobre 2017).

3.4 Collaborations

This research project is the result of fruitful collaborations with national and international research teams gathering ecotoxicologists, environmental chemists, ecophysiologicals, ecologists, parasitologists and microbiologists (Figure 1).

Field study	Experiment	Institution	Department	Coordinators	Analysis
<input type="checkbox"/>	<input type="checkbox"/>	METIS	Biogeochemistry	Aurélie Goutte	Pollutants
<input type="checkbox"/>	<input type="checkbox"/>	iEES Paris	Evolutionary ecophysiology	Clotilde Biard, Sandrine Meylan	Oxidative stress biomarkers
<input type="checkbox"/>	<input type="checkbox"/>	CEBC	Ecophysiology	Frédéric Angelier	Telomere
<input type="checkbox"/>		EDB	Aquatic ecology	Lisa Jacquin	Innate immune biomarkers
<input type="checkbox"/>		HEPIA	Ecology and aquatic systems	Raphaël Santos	Age, oxidative stress biomarkers
	<input type="checkbox"/>	CEREEP	Experimental platform	Beatriz Decencièrre	Experimental approach
	<input type="checkbox"/>	M2C	Microbiology	Fabienne Petit, Thierry Berthe, Yannick Colin	Gut microbiota



Figure 1. Main scientific and technical partners

3.5 Work contributions

Data collection

Fieldwork A. Goutte; F. Alliot; R. Santos; M. Chevreuil
Experimental setup & Manipulation **N. Molbert**; A. Goutte; A. Millot; S. Agostini

Chemical analysis

Contaminant analysis n¹ = 728 **N. Molbert**; F. Alliot; A. Desportes ; M. Khabou²
Lipid determination n = 217 **N. Molbert**
Isotope analysis n = 132 **N. Molbert**; M. Mendez; M. Mandeng-Yogo

Biological analysis

Age determination n = 132 **N. Molbert**; R. Santos
Gut microbiota analysis n = 27 Y. Colin; T. Berthe; **N. Molbert**
Oxidative status n = 657 **N. Molbert**; M. Leroux-Coyau ; R. Santos
Innate immune n = 149 L. Jacquin
Telomere analysis n = 267 F. Angelier; C. Ribout; **N. Molbert**

Statistical analysis **N. Molbert**, Y. Colin

¹Number of samples

²Supervisor of BSc student internship: Khabou Marwa. (2019, 2 months) Accumulation kinetics of polycyclic aromatic hydrocarbons within a host-parasite system.

I also held the position of teaching assistant for Master students for the course « Parasites in aquatic ecosystems » within the chair of “Aquatic Ecology” and, throughout these 3 years, the position of doctoral student representatives on the scientific council of the METIS laboratory.

4. Financial support

PhD funding was provided by the **Sorbonne University Environmental Transition Institute** (SU-ITE; [Figure 2](#)). **SU-ITE** also supported formations during this 3-years PhD (Animal experimentation and welfare). The project was financially supported by the **Seine-Normandy Water Agency** and the **PIREN-Seine program**. **METIS** and the **EPHE** contributed to the acquisition of experimental equipment.



Figure 2. Principal funders of the thesis project.

5. Abbreviations

CEBC: Centre d'Etudes Biologiques de Chizé

CEREEP: Centre de Recherche en Ecologie Expérimentale et Prédictive

WFD: Water Framework Directive

IUCN: Union Internationale pour la Conservation de la Nature

DEET: N,N-diethyl-m-toluamide

dwt: Dry Weight

GC-MS/MS: Gas chromatography coupled to tandem mass spectrometry

K_{ow} : Octanol/Water partition coefficient

LC-MS/MS: Liquid chromatography coupled to tandem mass spectrometry

LMM: Linear mixed models

LOD: Limit of Detection

LOQ: Limit of Quantification

OCP: Organochlorine Pesticides

OH-PAH: Hydroxylated metabolites of PAHs

PAH: Polycyclic Aromatic Hydrocarbons

PBDE: Polybrominated diphenyl ethers

PCB: Polychlorinated biphenyl

POP: Persistent Organic Pollutants

CYP 450: Cytochrome P450

DNA: Deoxyribonucleic acid

EROD: Etoxyresorufine-O-deethylase

HSI: Hepatosomatique Index

K: Fulton's body condition

MDA: Malondialdehyde

MFO: Mixed function oxidase

OTU: Operational Taxonomic Units

OXY: Antioxidant Capacity

PCR: Polymerase Chain Reaction

ROM: Reactive Oxygen Metabolites

ROS: Reactive Oxygen Species

RTL: Relative Telomere Length

SOD: Superoxide Dismutase

TBARS: Thiobarbituric acid reactive substances

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CHAPTER I.

GENERAL INTRODUCTION

« J'avais soif de toutes connaissances. Dans l'exubérance du monde sud-américain, la découverte des hommes et de l'immense nature fut jusqu'à mon dernier souffle, source de passion, d'émerveillement et de travail. »

Alcide d'Orbigny

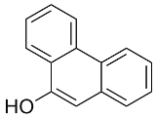
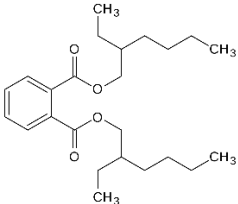
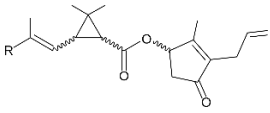
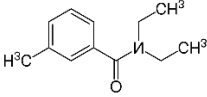
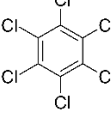
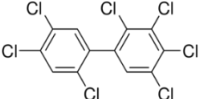
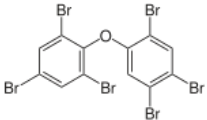
I – General introduction

1. Environmental contamination

At a global scale, freshwater ecosystems cover <1 % of the Earth's surface but shelter about 10 percent of all known aquatic species, including nearly half of the world's fish species (Balian et al., 2008). Although of fundamental importance to Humans, freshwater ecosystems are most impacted by anthropogenic-related pressures (overexploitation, flow modification, water pollution, invasion by exotic species, habitat degradation and climate change, Dudgeon et al., 2006) driving significant biodiversity losses, with an average two-thirds decline in less than half a century (WWF, 2018). Indeed, freshwater species suffer by far the worst losses in terms of relative changes in population abundance over time, close to four times greater than that of terrestrial populations (WWF, 2018). Due to their direct proximity to contamination sources, poor waste water treatment, storm water runoff, leaching from green spaces as well as agriculture and accidental discharge (Pierce et al., 1998), freshwater ecosystems are the ultimate receptacle for chemicals. They receive each year a growing number of new synthetic substances (Robin & Marchand, 2018), leading to a mixture of toxic effects. With more than 120 000 synthetic chemicals registered by the European Chemical Agency, countless organic compounds are potentially released into the environment (European Chemical Agency, 2017). In the last 20 years, environmental treaties, such as the Stockholm Convention on Persistent Organic Pollutants (POPs), have driven the global production and use of manufactured chemicals to shift from persistent, bioaccumulative and toxic compounds, such as the insecticide dichlorodiphenyltrichloroethane (DDT), to emerging contaminants ubiquitous in our daily life. In 2014, more than 40% of European rivers were ecologically impaired or threatened by a wide range of organic contaminants including pesticides, brominated diphenyl ethers (PBDEs, which are used as flame retardants in consumer goods) and polycyclic aromatic hydrocarbons (PAHs, which are released from fossil fuels) (Malaj et al., 2014; Table 1.1). Additionally, other synthetic compounds have gained widespread attention because of their unexpected or unknown biological activity and/or pseudo-persistence in aquatic environments. Over the past couple of decades, environmental plastic pollution has become a global threat to ecosystems and human health, encompassing a wide variety of polymers and additives with different chemical and physical properties (Hermabessiere et al., 2017). Among them, phthalate esters (hereafter phthalates) are a prominent group of organic micropollutants used as plastic additives (plasticizers) to provide flexibility in the manufacturing of plastic products such as polyvinyl chloride, and as a common additive in various consumer products (*i.e.*, cosmetics, paints, lubricants, adhesives, packaging) (OECD, 2004). Phthalates accounted for 65% of the

world consumption of plasticizers in 2017 (HIS Markit, 2018). Of equal concern, pyrethroids have taken an important place in the panoply of pesticides available, designed to have specific biological effects even at low concentrations, not only to control or fight insects in agriculture and residential environments but also to eradicate vectors of endemic diseases.

Table 1.1. Molecular structure, uses and sources of different groups of organic contaminants investigated in this thesis.

PAHs†		Incomplete combustion of organic materials (<i>e.g.</i> , coal, oil, petrol, and wood), oil spills
Phthalate esters†		Plasticizers: additives to make plastics more flexible and harder to break Non-plasticizers: in products such as lubricating oils, automobile parts, paints, glues, insect repellents, photographic films, perfumes, cosmetics and food packaging
Pyrethroids†		Insecticides used for household, commercial and farming applications. In medicine, pyrethroids are used against scabies and lice and to control endemic diseases such as malaria
DEET (N,N-diethyl-m-toluamide)		Insecticides. Active substance in many insect repellents
OCPs*† (Organochlorine pesticides)		Synthetic pesticides with vast application in the chemical industry, agriculture and mosquito control
PCBs*† (Polychlorinated biphenyl)		Use as heat exchange fluids, in the manufacturing of electrical equipment (transformers and capacitors), as additive agents in paint and plastics.
PBDEs† (Polybrominated diphenyl ethers)		Flame retardants in many manufactured goods, such as textiles, electronic equipment, plastics and furniture, to decrease their flammability

*Legacy POPs listed under the Stockholm Convention, banned from production and use, † listed as priority and hazardous substances

2. Fate of environmental contaminants in organisms

Once released in aquatic ecosystems, these contaminants can turn bioavailable for living organisms. In ecotoxicology, bioavailable contaminants are chemicals present in the environment that can be assimilated by living organisms and thus potentially toxic (McLaughlin & Lanno, 2013). When entering an organism, contaminants can bioaccumulate within

individuals (Figure 1.2A; Vives et al., 2005) and biomagnify across the food web (Figure 1.2B; Ren et al., 2017).

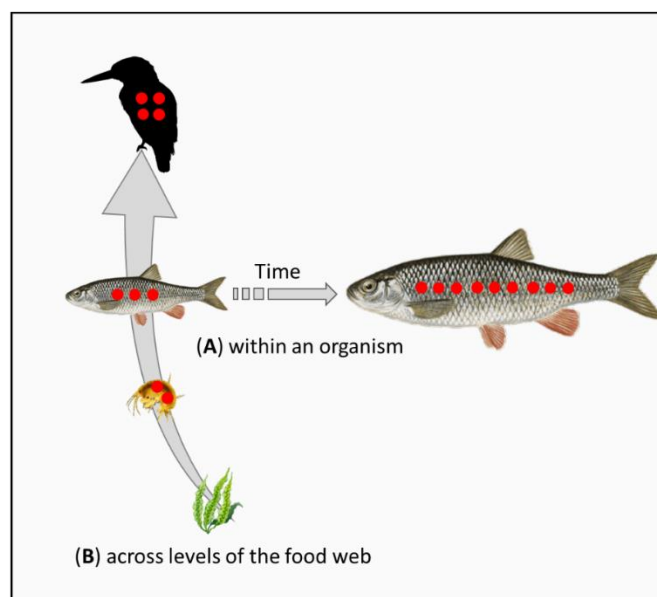


Figure 1.1. Processes of (A) bioaccumulation and (B) biomagnification in a freshwater ecosystem with a simplified food web. Red dots represent the pollutant load.

Both terms describe the transfer of contaminants from the external environment to the organism. Briefly, bioaccumulation occurs when contaminants are absorbed faster than they are eliminated and contaminants are biomagnified when they are transferred up the food web faster than they are transformed or excreted (*i.e.*, contaminant levels in consumer > than in the food sources). These processes are highly dependent on the physico-chemical properties of the chemical and the metabolic system resulting in a substantial variability of contaminant levels among organisms and tissues (Staples et al., 1997). This is indeed true for chemicals known as persistent organic pollutants such as OCPs, PCBs and PBDEs. Due to their highly lipophilic nature, and their slow biochemical degradation rates, they tend to accumulate in biota, reaching concentrations that may be harmful to health (Vives et al., 2005; Kelly et al., 2007; Yu et al., 2009). While most of them have been banned from use and production for decades, they still significantly contribute to the contamination of the different components of the environment and continue to be reported at sublethal levels in organisms (Ábalos et al., 2019).

In contrast to legacy organic pollutants, PAHs, phthalates and pyrethroids are readily metabolized by vertebrates (Barron et al., 1995). While this detoxification process facilitates the excretion of absorbed chemicals by the formation of water-soluble metabolites, it can lead to formation of toxic by-products, sometimes even more noxious than the parent molecule

(Wang et al., 2009; Figure 1.1), or generate reactive oxygen species (ROS) through redox cycling. This biotransformation process mainly takes place in the liver and is divided into two phases, whereby the aim is to increase the polarity of the compound to ensure the elimination from the organism (Matei et al., 2013). Phase I reactions are mediated by inducible cytochrome P450 dependent monooxygenases (CYP) and transform hydrophobic chemicals to more polar products *via* oxidation, hydrolysis, or similar reactions. The phase I metabolites can *i*) be directly excreted, *ii*) undergo a further conjugation reaction with biomolecules (phase II; *e.g.*, glutathione S-transferases, UDP-glucuronosyltransferases, N-acetyltransferases, sulfotransferases) that add polar functional groups to the Phase I by-products to produce more polar metabolites or *iii*) easily interact with endogenous functional molecules (*e.g.*, proteins, enzymes, nucleic acids) leading to an increase in toxicity (bioactivation).

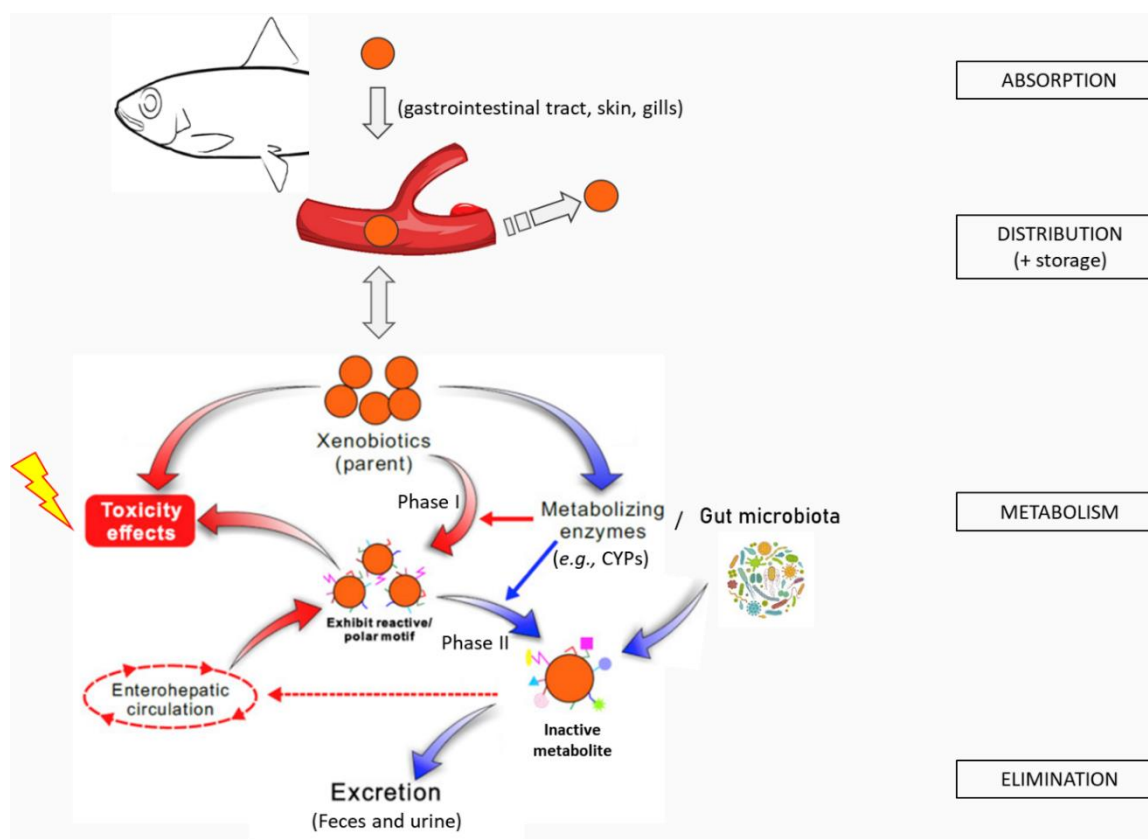


Figure 1.2. Simplified model of pollutants dynamic in fish body, from their absorption to their elimination/egestion. The main route of contamination exposure for fish is through dietary or gills uptake. Contaminant toxicokinetics involve assimilation of contaminants by the digestive tract (or by direct absorption from water through their gills and skin), transport into the bloodstream, distribution in internal organ/tissues, remobilization into the blood circulation and their excretion through feces, urine, eggs and parasites. Adapted from Wu et al., 2017.

While their excretion into urine efficiently ends up the exposure of the organism to the chemical, excretion in the bile may not always result in efficient elimination because an

enterohepatic circulation may occur (Franklin & Yost, 2000; Figure 1.1). This can result in the prolonged effects and persistence of some chemicals. This recirculation often involves the secretion of contaminant by-products in the bile and their hydrolysis by enzymes from the host or microorganisms in the gastrointestinal tract (gut microbiota). This deconjugation can result in the reabsorption of the chemical, if sufficiently soluble in lipids (*i.e.*, high octanol/water partition coefficient, $\log K_{OW}$; compounds that can be efficiently taken up by passive diffusion), as a pathway back in the portal circulation. Despite the importance of metabolites, only the concentrations of parent chemicals are most generally measured in biological and environmental matrices, which often result in an underestimation of contaminant exposure. To overcome this difficulty, the quantification of metabolites (will refer to the biotransformation products of organic contaminants in this thesis) has been applied, as much as possible, as a biomarker of exposure, although we are aware that much remains to be clarified regarding the effects of metabolites of organic contaminants upon wildlife, and that studies of the relationships between levels of metabolites and eco-physiological responses remain in their infancy (Fourgous et al., 2016). Contaminant (including their metabolites) distribution patterns in tissues are thus not only affected by the chemical structure or properties (molecular weight, metabolic rate: $\log k_M$, hydrophobicity: $\log K_{OW}$), but also by the biology and ecology of organisms (as generally measured by simple data such as sex, age and lipid content; metabolism and trophic position). However individual-related parameters may not be sufficient. Among ecological factors, interspecies interactions beyond the food web may be highly significant.

3. Effect of parasitism

Parasites are defined as any life form that depend upon another organism (host). This ecological interaction (parasitism) involves diverting a host's resources for one's own benefit (transmission, reproduction and survival). Thus, by definition, parasites do not have neutral effects on their hosts. In fact, parasites are ubiquitous components of biological systems, found virtually in all free-living organisms on earth (Strona, 2015). While parasites have been recognized as ecologically important by shaping animal communities and ecosystem function (Friesen et al., 2019a), there are also useful bioindicators of environmental changes. Parasites indeed provide information about the chemical state of their environment and have been used as effect indicators (*i.e.*, parasites abundance, composition and richness) and accumulation indicators for pollutants (*i.e.*, capacity to take up and accumulate substances) (Sures, 2004). The latter has been largely investigated in helminths of fish, including diverse groups of parasites (acanthocephalans, cestodes, nematodes and digeneans), from freshwater and marine

ecosystems (Sures et al., 2017). This remarkable ability to accumulate toxicants may therefore have implications for chemical levels in the host tissues, as suggested by a couple of studies on inorganic (see Sures et al., 2017 for review) and legacy chemicals (Vidal-Martínez et al., 2003; Brázová et al., 2012), with contrasting results on the pollutant burden in infected organisms depending on the host, parasite species and chemical under study (Jankovská et al., 2010).

Specifically, acanthocephalan parasites are endoparasitic worms that are trophically transmitted from an intermediate crustacean host to the intestine of a vertebrate definitive host (Kennedy, 2006). Their establishment in the final host is usually associated with mechanical damage and inflammation of the intestinal wall (Taraschewski, 2000), resulting from the penetration of the proboscis (*i.e.*, elongated appendage crowned with several rows of recurved hooks). Acanthocephalans have been reported to reduce the growth of their final hosts (Sakthivel et al., 2016; Silva-Gomes et al., 2017) and under conditions of very intensive infections, the worms may destroy the absorptive layers of the gut wall, completely obstruct the gastrointestinal tract and cause intestinal blockage (Aguiar et al., 2018). On the other hand, acanthocephalans could act as toxicant sinks and reduce the pollutant load of their host (Sures & Siddall, 1999). These endoparasitic worms are indeed able to concentrate environmental contaminants, reaching levels up to 2,700 times higher in acanthocephalans than in their host tissues (Sures & Siddall, 2003; Thielen et al., 2004; Filipović Marijić et al., 2013). Up to now, these studies have mainly focused on trace metals and more recently on PCBs (Brázová et al., 2012; Mille et al., 2020) but to date, very few studies considered the effects of emerging organic contaminants (Henríquez-Hernández et al., 2016; Mille et al., 2020) and none of them have simultaneously evaluated their fate and physiological consequences in host-parasite systems.

Studying the fate of chemicals 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 contaminants. We previously mentioned that chemical uptake and accumulation inside organisms may vary with the trophic level, length/age and lipid contents for hydrophobic molecules (as measured by the log K_{ow}) (André et al., 1990; Ko et al., 2018). Similarly, their molecular weight can affect the distribution of contaminants inside the studied system (Le et al., 2016). Acanthocephalan parasites lack a gastrointestinal tract, so that food assimilation mainly occurs through passive mechanisms (Goater et al., 2014). This physiological trait is likely to favor the absorption of smaller and more water-soluble molecules (Persson et al., 2007; Le et al., 2016), which are able to cross through the parasite tegument. At last, vertebrates and invertebrates strongly differ in their ability to metabolize xenobiotics (Livingstone, 1998), so that the chemical's susceptibility to

metabolic transformation ($\log k_M$) is expected to affect the fate of contaminants in host-parasite systems.

By interfering with the bioaccumulation of various chemicals, parasites may have a profound impact on the host resistance to pollution. However, interactions between hosts and parasites in polluted environments are complex and not well understood (Brown & Pascoe, 1989; Thilakaratne et al., 2007; Sánchez et al., 2016; Morrill et al., 2019). Their outcomes usually depend on the host-parasite combination as well as on the chemical investigated (Marcogliese & Pietrock, 2011), thus limiting our understanding of the ecology and evolution of host-parasite interactions. In fact, hosts and parasites can react differently to pollutants, influencing their mutual interactions. For instance, if the parasite is more susceptible than the host to environmental pollution or conversely, if chemicals negatively affect the host's survival, thereby compromising parasite transmission (Lafferty, 1997). Alternatively, the physiology of the parasite itself (*e.g.*, through their bioaccumulation capacity) may also change the stress response of the host to toxicants in different directions as both chemical exposure and parasite infection might interact in a synergistically (overall effect that is greater than the sum of individual effects), antagonistically (less than the sum of individual effects) or additive way (the sum of individual effects) (Coors & De Meester, 2008; Marcogliese & Pietrock, 2011). Interestingly, in recent years, counterintuitive findings have been reported in which parasites appear to be beneficial to their host under polluted conditions (Dautremepuits et al., 2002, 2003; Sánchez et al., 2016; Morrill et al., 2019), yet these studies exclusively focused on inorganic contaminants. Thus ecotoxicological studies carried out on infected organisms are likely to give rise to misleading interpretations when studies ignore parasites (Timi & Poulin, 2020).

4. Health effects of contaminant exposure and parasite infection

General biomarkers are known to respond to a variety of stressors, natural or anthropogenic, being thus appropriate to investigate the response of host-parasite system to contaminant exposure (Marcogliese & Pietrock, 2011). Additionally, a multi-biomarker approach in fish may be used as an integrated strategy, to take into account the diversity of environmental contaminants and the multiplicity of their effects, from molecular to individual levels.

4.1 Molecular and cellular levels

At the molecular and cellular level, the oxidative status is defined as the dynamic balance between the production of reactive oxygen species (ROS) and their detoxification through enzymatic and non-enzymatic defenses. When oxidative species production overcomes

antioxidant systems, oxidative stress arises. It can result in critical levels of oxidative damage to biomolecules (Soltani et al., 2019). 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 (Frank et al., 2011; Qu et al., 2015). 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, which generate relatively rapid response to invading pathogens. However, organic pollution has been linked to reduce immunocompetence of the host, increasing the likelihood of parasitic infection and diseases (Bols et al., 2001). Yet, studies simultaneously assessing both effects are rare and bring contrasting results on the joint effect of pollution and parasitism on immunity (Fatima et al., 2007; Le Guernic et al., 2016; Sueiro et al., 2017), depending on the parameters investigated, the parasite taxa (*i.e.*, direct toxic effects) and on the order that the organism experiences these stressors (Hoole et al., 2003). Consequently, the combined effects of parasitism and pollution on fish health are still poorly understood, especially in natural field conditions.

Through their adverse effects, environmental stressors (parasite infection and chemical exposure) may ultimately lead to fitness consequences, such as reduced animal's survival. Recently, telomeres have been recognized as robust molecular tools to predict life expectancy (Angelier et al., 2019; Whittemore et al., 2019). Located at the end of eukaryotic chromosomes, telomeres shorten through successive cell division and under the exposure to oxidative stress (Reichert & Stier, 2017). Beyond a critical telomere length, cell death occurs. Dead cells can accumulate in tissues leading to a decline in tissue function and aging effects. Independently, both parasite infection (Asgar et al., 2015; Karell et al., 2017; Stauffer et al., 2017) and chemical pollution (Blévin et al., 2016; Sletten et al., 2016) have been associated with shorter telomeres in wild organisms. While the underlying mechanisms still remain unclear, telomere length could provide valuable information on the potential fitness outcome of parasite infection in contaminated environments.

4.2 Organ and individual levels

At the organ level, recent studies have evidenced the role of the gut bacterial community (*i.e.*, microbiome) in resistance towards environmental pollutants. The metabolic capacity of gut microbiota being similar to that of the liver (Li & Jia, 2013), bacterial communities play key roles in the bioavailability and metabolism of various xenobiotics (Gaulke et al., 2016; Claus et

al., 2017). Perceived as a valuable physiological marker of stress, the microbiota has been increasingly studied in ecotoxicology (Evariste et al., 2019). According to previous studies, changing environmental conditions (contaminants) and ecological interactions (parasitism) shape the composition of gut microbiota, as well as their subsequent role in host physiology and immunity (reviewed in Butt & Volkoff, 2019). By inhibiting bacterial growth or inducing dysbiosis (*i.e.*, microbiome disruptions), environmental pollutants can affect the metabolic activity of gut microbiome, thereby modifying their toxicity and bioavailability in the body (Gaulke et al., 2016; Bagi et al., 2018; Zhao et al., 2019). Additionally, vertebrates' gut microbiota can be altered by the hosts' parasites. Intestinal parasites, such as acanthocephalan, can co-exist in the gut with microbial communities, and both are therefore capable of interacting with each other (Newbold et al., 2016; Fu et al., 2019; Ling et al., 2020). Such associations have the potential to affect host health as well as the gut microbiota and parasite populations themselves (Gaulke et al., 2019). For instance, parasite infection can promote or suppress bacterial taxa (Walk et al., 2010; Fu et al., 2019) and conversely, gut microbiota can influence parasite success or growth in the gut and mediate their impact on host physiology (Gaulke et al., 2019). Both stressors, parasites and pollutants, are then expected to have interactive effects on the composition and/or diversity of gut microbiota, with potential fitness consequences.

At the individual level, organ-somatic indices and body condition may provide further integrative information on the combined effects of chemical exposure and parasite infection on host health. Pollutants have been known to either stimulate or weaken the feeding behavior of fish (Kasumyan, 2001). In addition, parasites have typically been linked to hosts with poor body condition, as parasites are assumed to negatively affect hosts either directly by diverting resources and damaging tissues (Taraschewski, 2000; Aguiar et al., 2018) or indirectly by triggering costly immune responses and altering host behavior (Ranta, 1995; Fatima et al., 2007; Pegg et al., 2017). However, parasites have been reported to exert neutral, negative and positive effects on the host body condition (Santoro et al., 2013; Hursky & Pietrock, 2015; Maceda-Veiga et al., 2016), depending on the host–parasite interaction and underlying mechanisms driving condition–infection relationships (*e.g.*, host behavior, energy allocation, environmental conditions) (Sánchez et al., 2018). Still, the combined effect of parasitism and pollution on fish health remain poorly studied, although it would give valuable information on the potential costs and benefits of parasites in contaminated environments given that infection-induced changes to individual health can ultimately influence fitness and population viability.

5. Thesis objectives

In this thesis we investigated the fate and ecophysiological consequences of organic contaminants exposure in a host-parasite system composed of a freshwater fish, the European chub, *Squalius cephalus*, and its intestinal parasite, *Pomphorhynchus* sp. We decided to focus on six families of organic contaminants (PAHs, Phthalates, pyrethroids, PBDEs, OCP: organochlorine pesticides and PCBs: Polychlorinated biphenyl; [Table 1.1](#)), produced by human activities, except PAHs which have natural sources (*e.g.*, forest fires and volcanic activities), and listed as priority substances within the European Union Water Framework Directive (2013/39/UE; [European Commission, 2014](#)). The selected contaminants include long-term legacy POPs to allow for comparisons with previous studies.

The first objective of this thesis was to develop analytical methods to detect and quantify a large set of organic compounds (see above) as well as some of their main metabolites at trace concentrations in complex biological matrices of fish. There is indeed a need to routinely measure as many organic compounds as possible, including their metabolites. To date, most of the existing analytical methods applied to aquatic organisms are however restricted to a few groups of chemicals ([Teil et al., 2012](#); [Chatterjee et al., 2016](#); [Nagyová & Tölgyessy, 2019](#)), and none of them include both PAHs and plasticizers, which are primarily responsible for the poor chemical status of surface water bodies in Europe ([European Environment Agency, 2018](#)). This was achieved in **Article 1** (“*Multiresidue methods for the determination of organic micropollutants and their metabolites in fish matrices*”).

The second objective was to assess the influence of several ecological factors on the exposure to organic contaminants and their bioaccumulation inside organisms. This was completed in **Article 2** (“*Potential benefits of acanthocephalan parasites for chub host in polluted environments*”). Based on a correlative approach, I investigated the relationships between different groups of contaminants (parent molecules and their metabolites) and *i*) age, *ii*) length, *iii*) lipid content and *iv*) trophic level of chubs of the Marne River, France.

Additionally, it has been discussed throughout this Chapter that intestinal parasites, such as *Pomphorhynchus* sp., have the capacity to accumulate toxicants. While there is an extensive literature on inorganic pollutants and legacy POPs, little is known about the capacity of *Pomphorhynchus* sp. to accumulate emerging organic contaminants. In the same way, their fate and distribution in host-parasite systems have rarely been studied. The third objective evaluates the bioaccumulation of organic contaminants in *Pomphorhynchus* sp. and identifies potential underlying mechanisms to explain the distribution of different groups of organic chemicals in host and parasite tissues. Subsequently, the next objective was to assess the effect of parasite

infection on the pollutant burden of the fish host. This was achieved in **Articles 2** and **4** (“*Parasitism reduces oxidative stress and alters gut microbiota of fish host experimentally exposed to PAHs*”), based on a correlative and experimental approach.

Lastly, the physiological consequences of organic contaminants on interspecific relationship remain largely unexplored so that our last objective contributes at filling the gap knowledge. I evaluated the impact of both contaminant exposure and parasite infection by examining key traits linked to fish health across biological levels. This was completed in **Articles 2, 3** (“*Fish from urban rivers and with high pollutant levels have shorter telomeres*”) and **4**. Using a correlative and experimental approach, I investigated the relationships between environmental exposure to organic contaminants and several physiological and ecological relevant biomarkers in a fish host, the European chub, from wild populations of the Marne River, France. Specifically, I examined the relationships and causal link between the accumulation of several POPs, phthalates and pyrethroid pesticides and *i*) general health status (Fulton’s condition and hepatosomatic index), *ii*) gut microbiota, *iii*) innate immune responses (lysozyme and peroxidase), *iv*) oxidative status (pro- and antioxidants) and *v*) telomere length.

6. General predictions

We predicted that: **(1)** persistent organic pollutants would accumulate with age/length, higher lipid contents and trophic levels due to their slow biotransformation rate and their lipophilicity but not for metabolizable contaminants (**Figure 1.3A**) **(2)** that intestinal parasites would accumulate organic pollutants from their host, and preferentially those with high metabolic transformation rate ($\log k_M$), high solubility, and low molecular weight (**Figure 1.3B**), **(3)** infected hosts would have lower concentrations of organic pollutants in their tissues than uninfected ones due to the bioaccumulation process within parasites (**Figure 1.3C**), **(4)** exposure to organic pollutants would increase oxidative damage (ROMs and TBARS) and decrease antioxidant capacity in fish plasma (enzymatic and non-enzymatic), thereby shortening telomere length, **(5)** this effect would be reduced in infected chubs given the capacity of parasites to accumulate toxicants (**Figure 1.3D**); and **(6)** exposure to organic pollutants would impact bacterial diversity and composition of the host gut microbiota, with stronger effects for uninfected chubs compared to parasitized ones.

Although most material obtained is published or submitted in international scientific journals, this document does not simply present these articles as standalone chapters. We have decided to present a more synthetic vision of this thesis, answering to the previously enlighten research

questions and taking stock of the material and partial conclusions in all articles to draw a more comprehensive description of the overall work. Thus, our field study and experimental approach will be discussed simultaneously.

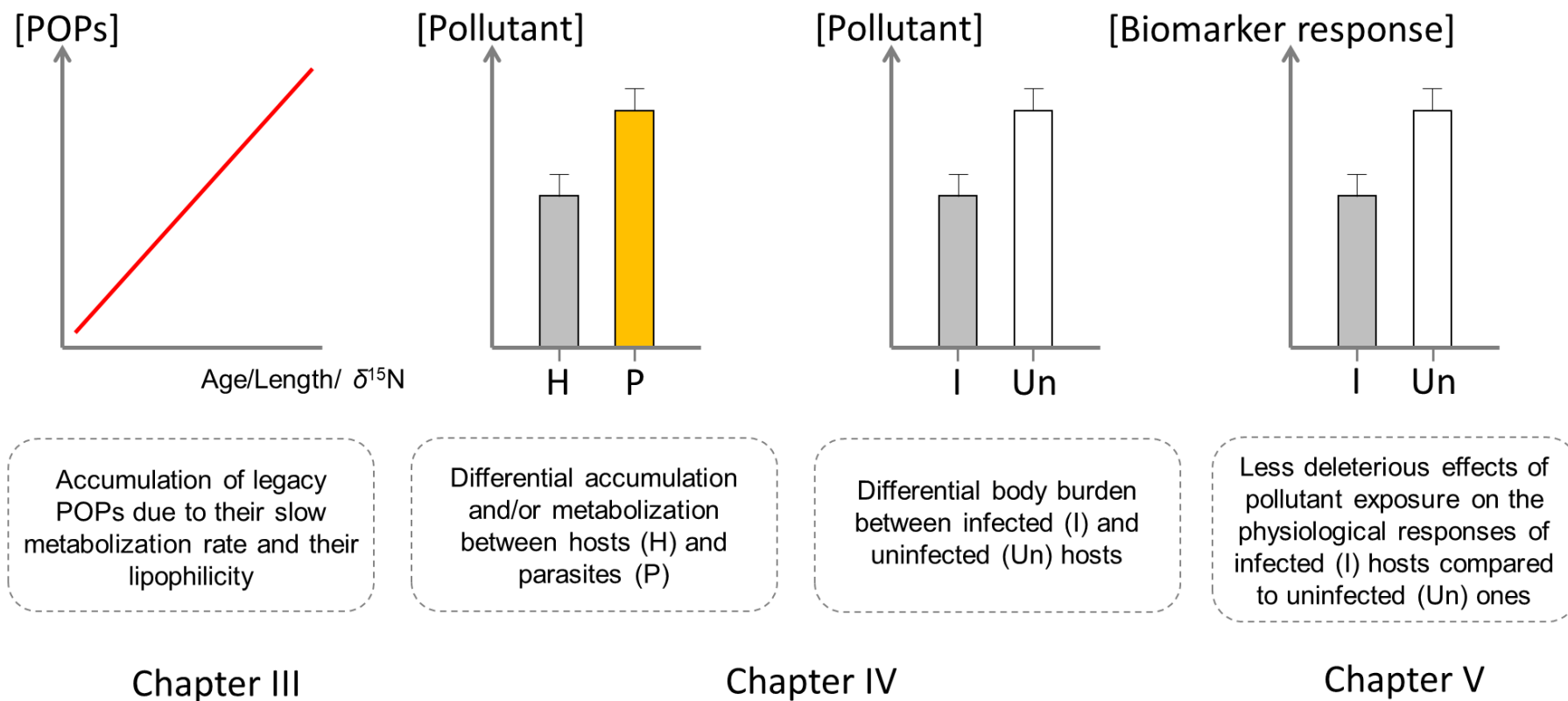


Figure 1.3. Overview of the main hypotheses and predictions in this thesis

CHAPTER II.

GENERAL METHODS

« Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we fear less. »

Marie Curie

II – General methods

1. Chub-acanthocephalan model

The European chub *Squalius cephalus* (hereafter “chub”; Linnaeus, 1758) is a freshwater cyprinid, among the most common and widespread fish species inhabiting European rivers. Chubs are gregarious animals living up to 20 years (Busst & Britton, 2014), sexually mature around 2-3 years for males and 2-4 years for females (Raikova-Petrova et al., 2012), and are considered as “least concern” species according to IUCN classification. Although chub is a non-migratory fish, they are able to cover short distances to spawn (up to 15 km; Fredrich et al., 2003). Contrary to other cyprinid species, chubs are not subject to manipulation such as stocking, which could interfere with natural processes (Bruslé & Quignard, 2001). As an omnivorous fish, they embrace a wide range of food items such as detritus, aquatic plants and invertebrates along the whole water column as well as small fish (Caffrey et al., 2008; Ünver and Erk’akan, 2011). Changes in their feeding habits occur along their growth, being mostly composed of small invertebrates for the juveniles to vegetation and aerial insects for the older ones, even adopting a piscivorous diet for the most mature specimens (Hellawell, 1971). These significant dietary shifts can therefore lead to higher pollutant load in older individuals because of bioaccumulation and biomagnification features especially for persistent organic pollutants (Merciai et al., 2014). Their distribution, abundance, large size, wide ecological niche, as well as the high environmental tolerance, especially to pollution, represent well suited characteristics to assess the effect and toxicity of anthropogenic pollution (Dragun et al., 2016). Over the last 20 years, chubs have indeed been recognized as model organisms for host-parasite systems in ecotoxicological studies (Sures et al., 1994; Sures & Taraschewski, 1995; Galli et al., 1998; Siddall & Sures, 1998; Sures et al., 1999; Sures & Siddall, 1999; Sures & Siddall, 2003; Sures et al., 2003; Filipović Marijic et al., 2013; Le et al., 2016; Figure 2.1).

Acanthocephalans are thorny-headed intestinal worms widely distributed in Europe, with indirect aquatic life cycles characterized by two host stages. They invariably use arthropods as intermediate hosts that get infected by consuming the eggs containing the acanthor larvae (Figure 2.2). The larvae grow into two different stages within the intermediate host: the acanthella and the cystacanth stages, only the latter being infectious for the definitive host. Many vertebrate species are known to serve as a definitive host for one or several species of acanthocephalan parasites (e.g., birds, mammals, fish, amphibians, reptiles, see Kennedy, 2006). Among them, *Pomphorhynchus laevis*, *P. tereticollis* use fish species especially the

chub, *S. cephalus*, as a preferred host in which the acanthocephalan parasite matures and reproduces (Kennedy, 2006).

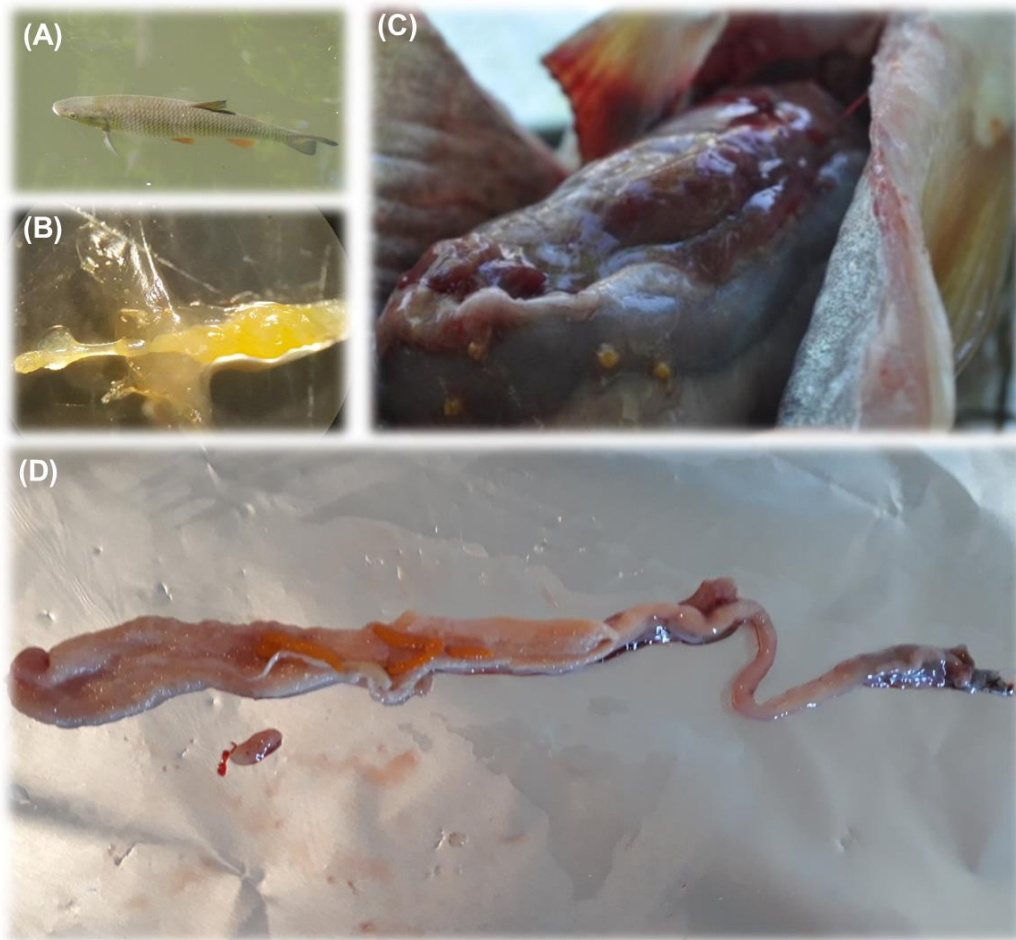


Figure 2.1. (A) the European chub, *Squalius cephalus*; (B) Intestinal acanthocephalan *Pomphorhynchus* sp.; (C) Outer view of the posterior intestine of chub with apparent proboscis of *Pomphorhynchus* sp. and (D) intestine cut open to show adults *Pomphorhynchus* sp. attached to the intestinal wall of chub. Photos: (A) Joe Dobinson (C) Aurélie Goutte et (C, D) Noëlie Molbert

However, due to their morphological similarities and a similar host spectrum, *P. laevis* and *P. tereticollis* can hardly be distinguished. Without molecular-based identification, parasites are thus reported throughout this thesis under the genus *Pomphorhynchus*. Their development is characterised by annual infection patterns (Kennedy, 2006). A growth phase of young acanthocephalans occurs immediately after establishing in the definitive host's intestine between the late autumn and winter months. Then, acanthocephalans mature in early spring and start reproducing during April to June. Their lifespan is approximatively of seven to eight months (Nachev & Sures, 2016). Importantly, *Pomphorhynchus* sp. inhabits the intestine of the chub where it can be exposed to whatever the host is ingesting, including contaminated food.

Therefore, this parasite–host system is considered to be a relevant model for determining whether *Pomphorhynchus* sp. could accumulate higher levels of organic contaminants compared with its host.

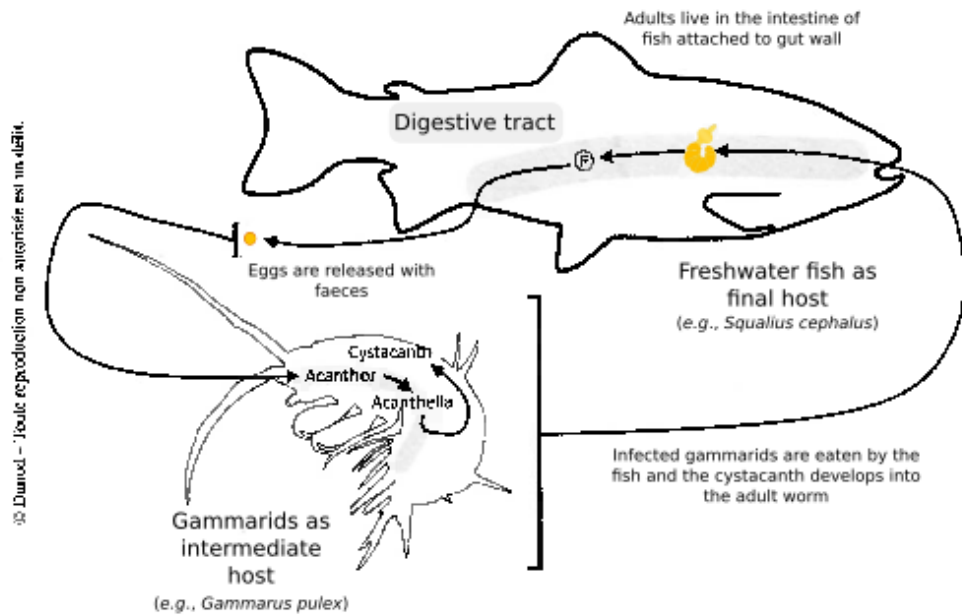


Figure 2.2. Schematic life cycle of *Pomphorhynchus* sp.

2. Data collection

Field approach: In September 2016, 118 chubs were caught by electrofishing, within twelve days to reduce temporal effects. Samplings were conducted on seven stations in the Marne River and its tributaries (Figure 2.3A), along a 290-km-long river stretch. The Seine basin currently accounts for 40% of national industrial activity (37% of its oil refining industry), 25% of national agriculture, and receives wastewater of 30% of the French population (Seine-Normandy Water Agency, 2011), therefore being a relevant example for studying the impact of anthropogenic activities on the environment. Fish from different sampling sites were considered as distinct populations given their relatively short-range movements (Penczak, 2006). Sites were selected based on their proximity to emission sources of organic pollutants (see Article 1): from agricultural (for organochlorine pesticides: OCPs) to urban (phthalates, PAHs and pyrethroids)/ industrial (PCBs and flame retardants: PBDE) areas.

Experimental approach: In January 2019, one population of chub 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 % of infected fish) and the levels of contamination is known (see Article 2).

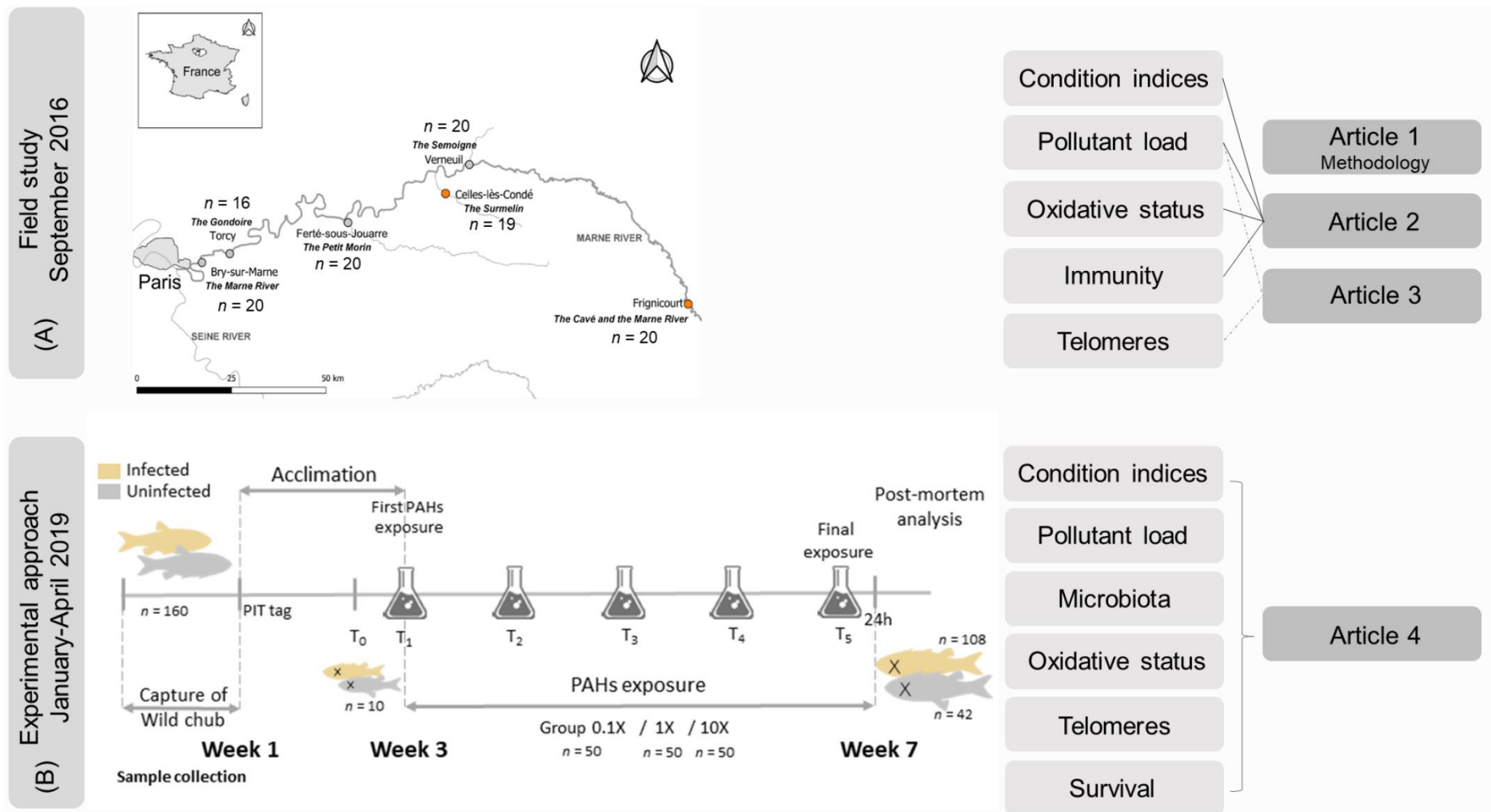


Figure 2.3. Data collection: **(A)** Field study conducted in September 2016 on wild chub collected from contrasting sites (urban: gray and agricultural: orange) on the Marne River and its tributaries, France. **(B)** Experimental approach conducted in January 2019 on wild chub electrofished on a tributary (Verneuil) of the Marne River. Numbers represent sample size.

Chub were selected on the basis of their specific body length ($n = 204$; mean \pm SD; 16.6 ± 2.68 g; 56.7 ± 28.3 cm), corresponding to immature individuals, electrofished within one week. 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 chubs (M222 , 80 mg L^{-1}) were then carefully tagged with a passive integrated transponder devices (8mm x 1.4mm 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 twenty 175-L tanks (80 x 60 x 42 cm) and were acclimated for 2 weeks before being exposed to PAHs. Chub being gregarious, a density of 10 fish per tank was respected 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 (Figure 2.4). Water temperature was progressively warmed up to indoor conditions during the acclimation period and kept constant during the experimental activity ($11.6 \pm 0.51^\circ\text{C}$). 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 during the experiment (mean \pm SD: pH 7.93 ± 0.09 , $187 \pm 5.21 \mu\text{S cm}^{-1}$, O_2 $9.60 \pm 0.29 \text{ mg L}^{-1}$, saturation $88.4 \pm 2.98 \%$).



Figure 2.4. Chubs in their respective tanks at the CEREEP-Ecotron facilities. Photo: Noëlie Molbert

A subsample of ten fish were sacrificed before the first contamination (T_0) to establish basal levels of PAHs in chub, assess their oxidative status and composition of gut microbiota (Figure 2.3B; Annex 5). Five-week experimental exposures to three levels of PAHs (0.1X, 1X and 10X) were then performed on three groups of fifty unparasitized and naturally infected fish. Treatment groups were assigned randomly among exposure tanks to allow the evaluation of the effects of parasite infection and PAHs exposure separately and in combination on biomarker responses. Each week (at T_1 , T_2 , T_3 and T_4), 10 fish exposed to 1X of PAHs were sacrificed for biomarker and pollutant kinetics (Figure 2.3B). Pollutant concentrations were prepared from a mixed solution of 16 PAHs dissolved in cyclohexane at $10 \text{ ng } \mu\text{L}^{-1}$ each, purchased from LGC standards. The exposure setup consisted of three environmentally relevant concentrations of a subset of PAHs (0.1X, 1X and 10X):

1. 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.
2. 1X concentration at 500 ng PAH/g of vegetal oil, representative of PAHs levels in wild invertebrate preys of chub (gammarids) captured in a river reaching good chemical status.
3. 10X concentration at 5,000 ng PAHs/ g of vegetal oil, corresponding to the highest level of PAHs quantified in wild chub (collected in 2016).

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 (Figure 2.5). 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 and at the end of the procedure, therefore the dose of PAHs diluted in vegetal oil was considered the administered dose. Experimental exposures were performed on sedated fish once per week (at T_1 , T_2 , T_3 , T_4 and T_5). During the experimental exposures, all fish were measured ($\pm 0.1 \text{ cm}$), weighted ($\pm 0.5 \text{ g}$) and checked for diseases. No visible health or behavioral changes were observed in chub during the acclimation and subsequent experimental periods. On the last week (T_5), fish were sampled 24-h after the last exposure to PAHs (Figure 2.3B; Varanasi et al., 1989).

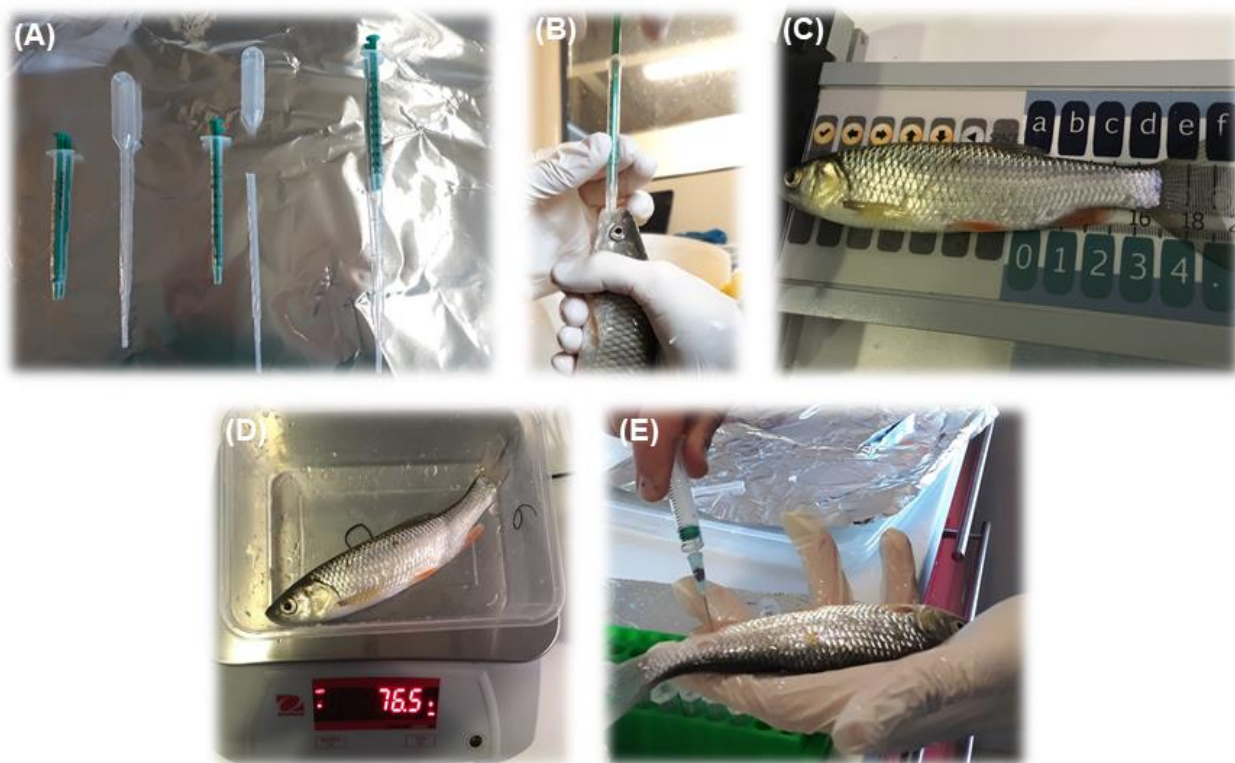


Figure 2.5. (A) Material used to inject contaminated oil, (B) injection of contaminated oil in the digestive tract of chub, (C) measuring, (D) weighing of chub and (E) blood sampling after five weeks of experimental activity. Photos: Noëlie Molbert

Whatever the approach, 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, 2,000 g, 4 °C). The plasma supernatant was separated out and stored at -20 °C for subsequent biomarker analyses. Following blood sampling, fish were euthanized with an overdose of the anesthetic agent (MS222, 1 g L⁻¹). Fish length (L_F ; ± 0.1 cm) and total weight (W_T ; ± 0.1 g) were recorded. Additionally, muscle tissues were collected in 2016 for isotopic analyses and muscle, liver, and stomach contents were dissected using stainless steel instruments. In 2019, muscle and liver were collected to assess PAH bioaccumulation and biotransformation in fish. In 2016 and 2019, the liver was weighted (W_L ; ± 0.01 g) and frozen at -20 °C until further processing. Chub intestine and body cavity were examined for the presence of acanthocephalan parasites. Whole intestine were aseptically dissected and all parasites were found within the host intestine. They were carefully removed from the chub intestine and counted to evaluate their prevalence (the percentage of infected chub) and intensity (parasite number per infected host individuals, (Bush et al., 1997)). Individual parasites of *Pomphorhynchus* sp. from the same host were pooled for chemical analysis, if applicable. From 0.99 to 500 mg of freeze-dried and 0.001 to 0.143 g of

wet weight parasite biomass was recorded per fish, in 2016 and 2019, respectively. Additionally, microbial samples were collected in 2019 by scrapping away the intestinal wall with sterile scalpel blades and then frozen at -80 °C until DNA extraction. Note that microbial samples were free of faecal material. Gut microbiota samples were pooled (n = 23 pools of three individuals) based on the respective exposure tanks of fish host and their infection status, to obtain enough biological material to carry out DNA extraction. T₀ (n = 3) were used to control for experimental conditions susceptible to affect the bacterial community (commercial food), group exposed to PAH-0.1X (n = 10) to control for PAHs exposure and 10X (n = 10) to evaluate the effect of both parasite infection and PAHs exposure.

3. Chemical analysis and properties

In 2016, a total of 68 compounds were quantified, including 21 listed as priority and hazardous substances ([European Commission, 2014](#)). They were selected because of their high usage rates and ubiquity in aquatic environments. Among them, 48 parent compounds belong to 6 families of organic contaminants: phthalates, PAHs, PCBs, PBDEs, OCPs, and insecticides (pyrethroids and DEET), as well as 20 of their metabolites: 7 phthalate monoesters, 11 hydroxylated metabolites of PAHs and 4 pyrethroid metabolites (see [Table 2.1](#)). For the experimental approach, we selected 16 PAHs recognized as a main cause of deterioration of aquatic ecosystems, especially in Europe ([Malaj et al., 2014](#); [European Environment Agency, 2018](#)) and listed as priority pollutants by the United States Environmental Protection Agency.

3.1 Chemical properties

Chemical properties such as the hydrophobicity ($\log K_{OW}$; the octanol/water partition coefficient is defined as the ratio of a chemical's concentration in the octanol phase to its concentration in the aqueous phase of a two-phase octanol/water system), metabolic biotransformation rate ($\log k_M$) and the molecular weight were used to investigate the fate and distribution of organic pollutants in the host-parasites system. $\log K_{OW}$ were obtained using the free online “Molinspiration Cheminformatics” web services (<http://www.molinspiration.com/>) and metabolic biotransformation rate ($\log k_M$) values were obtained with the BCFBAF module in EPIWEB 4.137 ([US EPA, 2012](#)).

Table 2.1. List of organic micropollutants, parent molecule (grey) and some of their metabolites (orange), quantified in this thesis.

Emerging pollutants		Legacy pollutants	
Polycyclic Aromatic Hydrocarbons (PAHs)		Organochlorine Pesticides (OCPs)	
naphthalene*†		pentachlorobenzene*	PeCB
acenaphthylene†		hexachlorobenzene*	HCB
acenaphthene†		lindane	γ-HCB
fluorene†		dichlorodiphenyldichloroethylene	p,p'-DDE
phenanthrene†		Polychlorinated biphenyls (PCBs)	
Anthracene*†		PCB-28	
fluoranthene*†		PCB-52	
pyrene†		PCB-101	
benzo-a-anthracene†		PCB-118	
chrysene†		PCB-138	
benzo-b-fluoranthene*†		PCB-153	
benzo-k-fluoranthene†		PCB-180	
benzo-a-pyrene*†		Polybrominated diphenyl ethers (PBDEs)	
indeno-1,2,3-cd-pyrene*†		BDE-28*	
dibenzo-a,h-anthracene†		BDE-47*	
benzo-ghi-perylene*†		BDE-99*	
By-products		BDE-100*	
1-hydroxynaphthalene†	1-OH-Nap	BDE-153*	
2-hydroxynaphthalene†	2-OH-Nap	BDE-154*	
2-hydroxyfluorene†	2-OH-Flu	N,N-diethyl-meta-toluamide (DEET)	
3-hydroxyfluorene†	3-OH-Flu		
9-hydroxyfluorene†	9-OH-Flu		
1-hydroxyphenanthrene†	1-OH-Phe		
2-hydroxyphenanthrene†	2-OH-Phe		
3-hydroxyphenanthrene†	3-OH-Phe		
4-hydroxyphenanthrene†	4-OH-Phe		
1-hydroxypyrene†	1-OH-Pyr		
6-hydroxychrysene†	6-OH-Chr		
3-hydroxybenzo[a]pyrene†	3-OH-BaP		
Phthalates		Pyrethroid pesticides	
dimethyl phthalate	DMP	bifenthrin,	
diethyl phthalate	DEP	permethrin	
n-butyl benzyl phthalate	BBP	phenothrin	
di-n-butyl phthalate	DnBP	cyfluthrin	
di-iso-butyl phthalate	DiBP	cypermethrin*	
di-2-ethylhexyl phthalate*	DEHP	fenvalerate	
di-n-octyl phthalate	DnOP	deltamethrin	
By-products		By-products	
mono-methyl phthalate	MMP	3-2,2-dichlorovinyl-2,2-dimethyl-	cis-DCCA
mono-ethyl phthalate	MEP	1-cyclopropane	trans-DCCA
mono-iso-butyl phthalate	MiBP	4-fluoro-3-phenoxybenzoic acid	4-FPBA
mono-n-butyl phthalate	MnBP	3-phenoxybenzoic acid	3-PBA
mono-benzyl phthalate	MBzP		
mono-n-octyl phthalate	MnOP		
mono-2-ethylhexyl phthalate	MEHP		
mono-2-ethyl-5-oxohexyl phthalate	MEOHP		
mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP		

*Listed as priority substances under the Water Framework Directive (2000/60/EC); † compounds quantified in every publications of the present thesis.

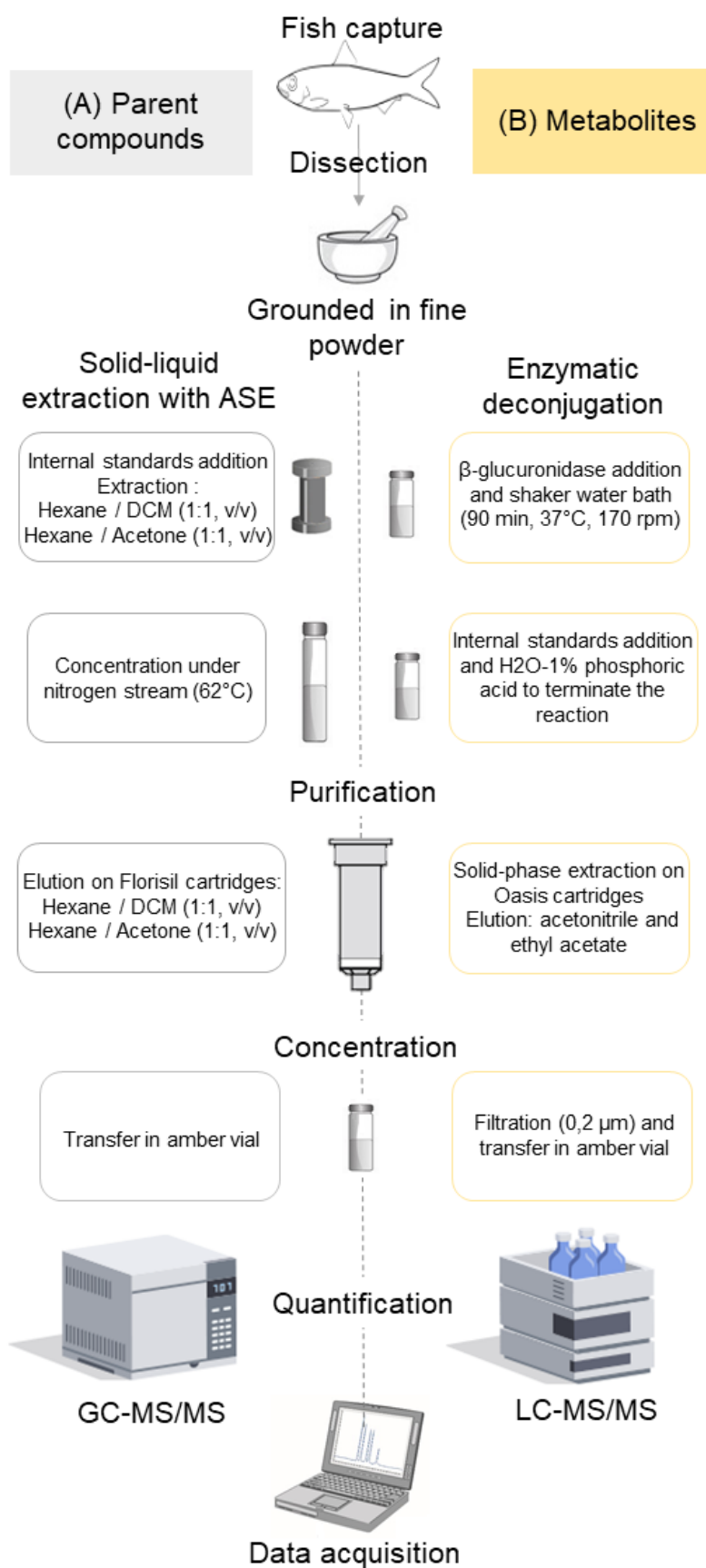


Figure 2.6. Overview of the developed protocols for the quantification of (A) parent molecules of organic compounds and (B) their metabolites.

3.2 Parent molecules

A total of 48 molecules were quantified in fish matrices and parasite tissues, and their analyses were conducted according to recently validated protocols and methods (see **Article 1**²). The main analytical steps are shown in [Figure 2.6A](#). Briefly, freeze-dried and homogenized samples (0.1 – 0.5 g for fish matrices and 0.02 g for parasite tissues) spiked with a mixture of all internal standards were extracted by accelerated solvent extraction (ASE). Extracts were then purified using Florisil cartridges, concentrated and analyzed by gas chromatograph coupled to a mass spectrometer (GC-MS/MS). Recoveries and repeatabilities were within the acceptable range and below the acceptable limit of 20%, (see **Article 1** for more details). The accuracy of the method was also within acceptable range, as our measurements were in good agreement with certified values (certified reference materials, Pacific herring). Procedural blanks were performed following the same treatment steps as the samples.

3.3 Metabolites

A total of 25 metabolites of organic pollutants were quantified in fish matrices (bile and liver) and parasite tissues, and their analyses were conducted according to recently validated protocols and methods (see **Article 1**). The main analytical steps are shown in [Figure 2.6B](#). Briefly, ammonium acetate was added to freeze-dried and homogenized samples (0.1 g for fish matrices and 0.02 g for parasite tissues). β -glucuronidase was added to the mixture, placed in a shaking water bath for the deconjugation step and spiked with a mixture of all internal standards ([Mazéas & Budzinski, 2005](#)). Indeed, the metabolites studied (*e.g.*, OH-PAHs) are present in their free form or more commonly conjugated with glucuronide, sulfate, or glutathione groups ([Varanasi et al., 1989](#)). The supernatant was extracted on solid-phase extraction cartridges. Extracts were then concentrated and filtered before analyzes by liquid chromatograph coupled to a mass spectrometer (LC-MS/MS). Recoveries and repeatabilities were within the acceptable range and below the acceptable limit of 20%, (see **Article 1** for more details). The accuracy of the method was also within acceptable range, as our measurements were in good agreement with certified values (SRM-3672 human urine).

4. Lipid determination

² **Molbert**, N.; Alliot, F.; Santos, R.; Chevreuil, M.; Mouchel, J.M.; Goutte, A. Multi-residue methods for the determination of organic micropollutants and their metabolites in fish tissues. *Environ. Toxicol. Chem.* **2019**, 38, 1866–1878. (doi:10.1002/etc.4500).

For each sample, a freeze-dried aliquot of 0.1 g of muscle, liver and stomach content was analyzed to determine its lipid content. Briefly, fish tissues were sonicated for 15 min with 2 × 5 mL of hexane:isopropanol (60:40, v/v) and then centrifuged (10 min, 2500 rpm; Labadie et al., 2010). The supernatant was deposited on a Vivaclear centrifugal filter (0.8 µm; Sartorius) and rinsed twice with 0.2 mL of hexane:isopropanol (60:40, v/v). Extracts were evaporated under a nitrogen stream (62 °C), and the residue was weighed to the nearest 0.1 mg. The same method was applied for parasite tissue (0.05 g), and solvent volumes were adjusted proportionally to the aliquot.

5. Age determination

Scales were collected in 2016 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 (red marks on Figure 2.7) on the scales (Bagenal, 1978). 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.

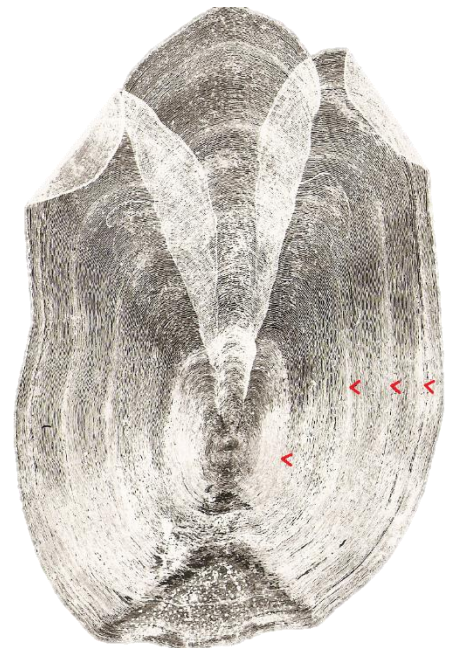


Figure 2.7. Scale of Northern pike, *Esox lucius*, showing four annuli (red mark). Photo: Francis Dauba

6. Stable isotopes

Isotopic measurements were performed on 0.2–0.25 mg of freeze-dried and powdered muscle samples of chub collected in 2016 (n = 113). 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). Stable isotope abundances are expressed as the deviation from international standards in parts per thousand (δ , ‰) as follow: $\delta X\text{‰} = [(R_{\text{ech}} / R_{\text{standard}}) - 1] * 1000$, with X corresponding to ^{13}C or ^{15}N and R representing $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratios. Atmospheric N_2 and Pee Dee Belemite were used as standards for nitrogen and carbon, respectively. The analytical precision was found to be 0.1‰ and 0.7‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. We used $\delta^{15}\text{N}$ as a proxy of trophic position (Cabana & Rasmussen, 1994). Additionally, to compare the isotopic niche space between infected and uninfected chubs we calculate standard ellipses, which represent the isotopic niche size of roughly 40% of individuals within the groups (Jackson

et al., 2011). This method accounts for core isotopic niche areas, being less sensitive to sample size than convex hull methods, allowing more robust comparisons among groups (infected vs uninfected) (Syväranta et al., 2013). Using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, we calculate the standard ellipses for each group to compare their size and overlap in each sampled site. We used the corrected version of the standard ellipse area (SEA_C) to control for sample sizes. To compare isotopic niche space between groups we compute the Bayesian estimate of the standard ellipse area (SEA_B). All statistics were generated with the SIBER package in R (Jackson et al., 2011).

7. Biomarker analysis

We used a multibiomarker approach in fish as an integrated strategy, to take into account the diversity of the contaminants quantified in this thesis and the multiplicity of their effects. It comprehends general biomarkers to evaluate the host condition and its metabolic efficiency, as well as the EROD activity, innate immune, oxidative status and telomere length.

7.1 General biomarkers

General biomarkers of fish health, the Fulton's condition factor (K) and the hepatosomatic index (HSI), were calculated in both approaches according to the following equations, respectively:

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

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

Where W_T , W_L and L_T represent the total wet weight of chub (g), the wet weight of the liver (g) and the total length (cm), respectively. A higher ratio indicates a better condition (K) and a higher energetic status/metabolic efficiency (HSI).

7.2 Chemical exposure

Measurement of ethoxyresorufin-O-deethylase (EROD) activity in fish is a well-established *in vivo* biomarker of exposure to organic pollutants, especially to halogenated (PCBs) and PAHs compounds. Through the induction of key biotransformation enzymes (cytochrome P450 1A monooxygenase), EROD is a highly sensitive indicator of contaminant exposure in fish. EROD activity was measured in chub liver ($n = 51$) collected in 2016, following Flammarion (1998). Briefly, the liver homogenates were centrifuged at 9 000 g for 15 min at 4 °C, the supernatant (S9 fraction) was extracted and resuspended in a buffer solution (phosphate-buffered saline, PBS) according to the amount of protein. A colorimetric method, the Bicinchoninic Acid Protein assay (Stoscheck, 1990), was used for protein quantitation.

7.3 Innate immune system

The enzymatic activities of peroxidase and lysozyme are two commonly used biochemical markers to evaluate innate immune responses (Guardiola et al., 2014), which play a major role in fish defence mechanism (Uribe et al., 2011). 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 & Roth, 1997) at Bordeaux Sciences Agro (Gradignan, France) using 5 μL of fish plasma collected in 2016. The absorbance was recorded at 450 nm. Following the procedure described by Ellis (1990), lysozyme activity was measured using fish plasma (15 μL) and 150 μL of a solution of *Micrococcus lysodeikticus* suspension (20 mg 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. Details are presented in **Article 2**.

7.4 Oxidative status

The oxidative status of an animal is defined as the balance of pro-oxidant compounds and antioxidant defenses. When oxidative species production overcomes antioxidant systems, oxidative stress arises, which can result in critical levels of oxidative damage to biomolecules (Soltani et al., 2019).

7.4.1 Antioxidants

Based on their activity, antioxidants are classified as enzymatic (convert oxidized metabolic products in a multi-step process) or non-enzymatic (intercept and terminate free radical chain reactions) antioxidants. 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 (*e.g.*, bilirubin, uric acid, vitamin C and E, albumin), after 1:100 dilution of 5 μL of plasma sample collected in 2016 and 2019 (Table 2.3). OXY concentrations³ were expressed as mM HOCl neutralized and details of the procedure are presented in **Article 2**. The activity of superoxide dismutase (SOD, enzymatic antioxidant;

³ Calibrations were achieved for both OXY and d-ROMs tests by measuring the absorbance (540 nm; iMark microplate reader, Bio rad California, USA) of a standard solution provided with the kit. Intra-assay and inter-assay variations for ROMs and OXY are described in **Articles 2** and **4**.

detoxifies toxic superoxide anion radicals) was measured to assess the capacity to maintain the redox status of fish. The SOD activity in erythrocyte was assayed following the colorimetric method of [Gagné \(2014\)](#) adapted for increased sensitivity. Results are expressed in units ([Table 2.3](#)), according to the protocol of [McCord and Fridovich \(1969\)](#), where one unit of SOD corresponds to the amount of sample causing 50% inhibition of the colorimetric reaction.

7.4.2 Pro-oxidants

The d-ROMs test (MC003, Diacron International, Grosseto, Italy) measures the concentration of reactive oxygen metabolites (ROMs) as a measure of oxidative damage in 4 μ L plasma sample collected in 2016 and 2019 ([Table 2.3](#)). Hydroperoxides are the main molecules measured by the d-ROMs ([Costantini, 2016](#)), which derive from the oxidation of biomolecular substrates and act as precursors of end-products of lipid peroxidation ([Beaulieu & Costantini, 2014](#)). ROMs concentrations were expressed as mM H₂O₂ equivalents and details of the procedure are presented in **Article 2**. Additionally, we evaluated secondary lipid oxidation products with the TBARS assay (Thiobarbituric acid reactive substances), which consists in the reaction of malonaldehyde (MDA, produced due to the oxidation of fatty acids) with thiobarbituric acid in acidic conditions and at a higher temperature (85 °C). TBARS assay provides a general quantification of oxidative damage molecules ([Beaulieu & Costantini, 2014](#)). Briefly, the erythrocyte suspension, obtained after osmotic shock, was added to a phosphate-buffered saline solution (PBS, 10 mM) before extraction of the supernatant (S9 fraction) following [Ohkawa et al. \(1979\)](#) adapted for fluorescence microplate assays. The results of the assay are expressed in μ mol of MDA equivalents ([Table 2.3](#)) for 103 samples.

7.5 Telomere

Recently, telomeres have been recognized as robust molecular tools to predict life expectancy ([Angelier et al., 2019](#); [Whittemore et al., 2019](#)). Telomere length was determined in fins by quantitative PCR (qPCR; BioRad CFX 96, Bio-Rad USA) according to [Petitjean et al. \(2020\)](#), adapted for the European chub. Briefly, fin samples collected in 2016 and 2019 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 some samples were excluded due to poor DNA quality (total sample analyzed: n = 98 and n = 111 for 2016 and 2019, respectively, [Table 2.3](#)). The control single-copy gene Recombination Activating Gene 1 (RAG-1) was selected and amplified using specific primers ([McLennan et al., 2019](#)) designed for the European chub. For detailed descriptions of the telomere and RAG-1 primers as well as

the qPCR conditions see **Articles 3 and 4**. All samples were randomly distributed across the PCR plates. Telomere length (T/S ratio) was expressed relative to the internal single gene control (RAG-1). Amplification efficiencies and the average inter-plate variation of the T/S ratio values are presented in **Articles 3 and 4**.

Telomeres, the terminal caps of eukaryotic chromosomes, protect the genome during cell division. Telomeres naturally shorten through successive cell division and thus with advancing age (Hatakeyama et al., 2016). Given that wild chubs exhibit an age- (or length-) related decline in telomere length (Figures 2.8A and 2.8B) in this thesis, the relative telomere length (RTL) was first corrected with age or body size (RTLc) to account for differences between individuals.

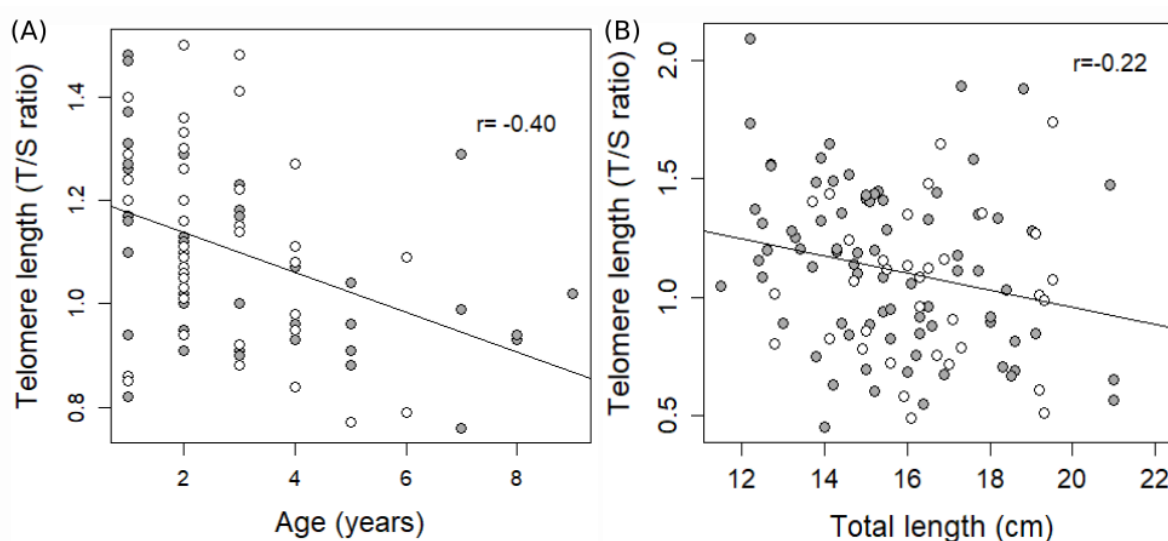


Figure 2.8. Relationship between telomere length (T/S ratio) and age/body size of chub (**A**) under natural settings in 2016 and (**B**) after five weeks experimental exposure to PAHs in 2019.

8. Data analysis, contaminant levels and relative contributions

The levels of contaminants (Σ Phthalates, Σ PAHs, Σ Pyrethroids, Σ OCPs, Σ PCBs, Σ PBDEs, Σ phthalate metabolites, Σ hydroxy-PAHs, and Σ pyrethroid metabolites) are expressed in nanograms per gram of dry weight (ng g^{-1} of dwt) throughout the manuscript. Consistently across the different fish matrices sampled in 2016, contaminant profiles were characterized by high levels of phthalates, pyrethroid pesticides and PAHs (Figure 2.9A). These three families of organic compounds contributing up to 80-90% of the total contaminant burden in chubs, were also found to predominate in several profiles of different fish species (Teil et al., 2012; Kampire et al., 2015). Even though those contaminants are rapidly metabolized by vertebrates, they are considered to have “pseudo-persistence” because of their continual input to the aquatic

environment, compared to most legacy POPs subjected to regulations (*e.g.*, PCBs and OCPs; Stockholm convention). A greater proportion of PAHs was measured in the digestive tract compared to other fish tissues, which suggests that the primary source of PAHs in fish is the consumption of contaminated food or the ingestion of PAHs adsorbed onto the particulate phase and sediments. This result justifies the injection of PAHs directly into the fish stomach during the experimental approach. Additionally, liver samples were collected to assess the levels of metabolites. The liver was selected as it is the primary organ of biotransformation. Importantly, in 2016, levels of metabolites were comparable to those of parent compounds within chub liver (*e.g.*, phthalates: $978 \pm 988 \text{ ng g}^{-1}$ of dwt and phthalate metabolites: $1,031 \pm 989 \text{ ng g}^{-1}$ of dwt; [Figure 2.9B](#)). This result strengthens the usefulness of metabolite analyses to better assess the impacts of currently-released chemicals on wildlife.

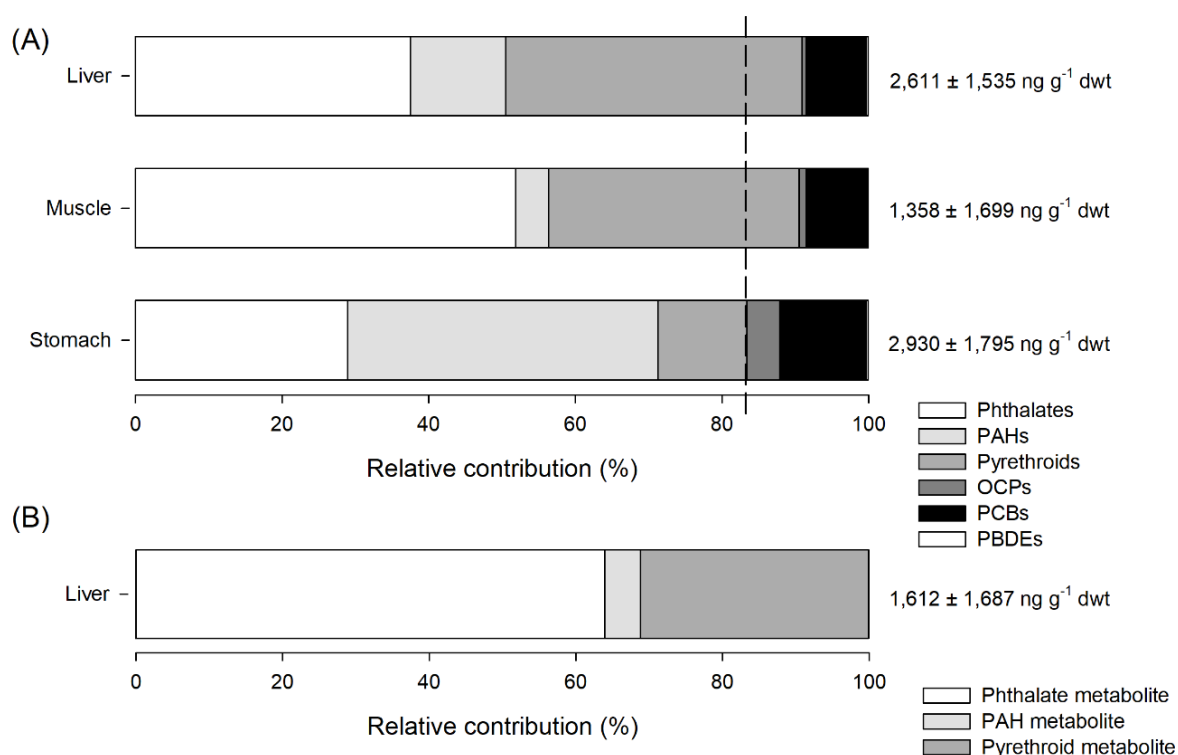


Figure 2.9. Relative contribution (%) of **(A)** parent organic contaminants and **(B)** some of their metabolites in different matrices of the European chub in 2016. Numbers represent the mean \pm standard error of total contamination per matrix. Sample size: **(A)** $n = 118$ for muscle, $n = 35$ for stomach contents and $n = 26$ for liver, **(B)** $n = 93$ for liver.

At the same sampling site (*i.e.*, Verneuil), fish collected in September 2016 and January 2019 significantly differ in their tissue levels of PAHs ($F_{(1,36)}=212.3$, $p<0.001$) ([Figure 2.10](#)). Analysis of fish tissues at the beginning of the experiment (T_0) revealed high levels of PAHs in

chub muscle ($n = 10$; $399 \pm 78.4 \text{ ng g}^{-1} \text{ dwt}$) and liver ($n = 5$; $784 \pm 159 \text{ ng g}^{-1} \text{ dwt}$), ten times higher than the levels reported from chubs collected in 2016. This higher contamination could not be explained by fish length given that chubs were smaller ($18.8 \pm 2.39 \text{ cm}$) in 2019 compared to individuals sampled in 2016 ($24.2 \pm 7.98 \text{ cm}$; t.test, $t=2.472$, $p<0.01$). It is likely that the collection period may explain the observed variation in PAHs contamination. In fact, the hydrological conditions and the pollution sources are the main factors causing fluctuation of PAHs in the environment (Ohiozebau et al., 2016).

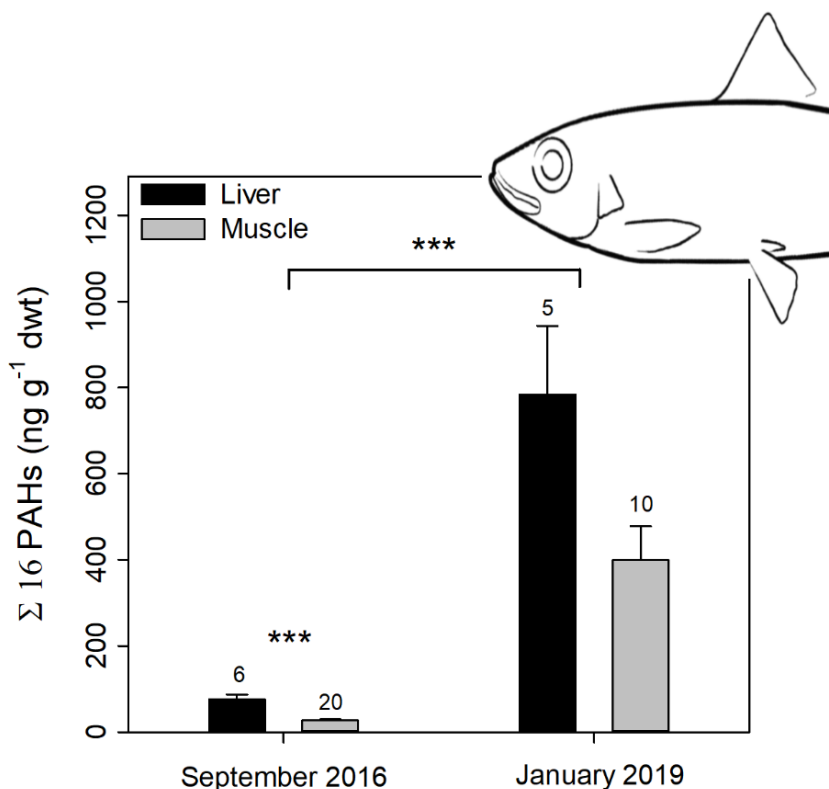


Figure 2.10. PAHs levels in different tissues (muscle and liver) of the European chub, *Squalius cephalus*, collected in 2016 and 2019 (at T₀). Numbers represent sample size. * represents a significant difference (***: $p<0.001$).

For specific statistical analyses, please refer to the articles attached at the end of the manuscript. For unpublished data (*i.e.*, EROD, SOD, TBARS, isotopic niche⁴), results are described in the manuscript. We used a Pearson correlation matrix to assess potential collinearity between the different families of organic contaminants (Table 2.2). Due to a high degree of correlation between the variables, we had to reduce the number of contaminants in further statistical analyses, and we assessed their effects on biomarker responses as the sum (Σ) of each

⁴ Results on isotopic niche will be published as a short communication

contaminant family separately and as the sum of total pollutant load (\sum Phthalates + PAHs + OCPs + PCBs + PBDEs + Pyrethroids) to evaluate the cocktail effect.

Table 2.2. A correlation matrix across the 6 families of contaminants under study, using Pearson's correlation coefficients (r). Bold values represent a significant correlation at $\alpha = 0.05$. The upper part indicates the strength and direction of the relationship (r) and the lower part the *p*-value.

	Phthalates	PAHs	Pyrethroids	OCPs	PCBs	PBDEs
Phthalates	-	0.41	-0.23	-0.02	0.03	0.34
PAHs	<0.001	-	0.02	-0.13	-0.03	0.24
Pyrethroids	<0.05	0.815	-	-0.15	0.07	-0.16
OCPs	0.821	0.209	0.143	-	0.27	0.12
PCBs	0.788	0.767	0.469	<0.01	-	0.05
PBDEs	<0.001	<0.05	0.113	0.256	0.652	-

9. Synthesis

For a better understanding, [Table 2.3](#) summaries all morphological, physiological and chemical data used in this thesis. It provides a description of all the parameters, their interpretation, the biological sample used (if relevant) as well as the related articles (see the **Appendix** at the end of the manuscript) in which the different data have been exploited.

Table 2.3. Description of the morphological, physiological and chemical parameters exploited in this thesis. (M: muscle, L: liver, S: stomach content and P: parasite)

	Variable	Matrix	Use	Variable description	Data		# Article			
					2016	2019	1	2	3	4
<i>Ecological and physiological markers</i>	Fulton's condition factor	-	Evaluation of the general condition	High ratio indicating healthier condition						
	Hepatosomatic Index	Liver	Energetic status and/or metabolic efficiency	High ratio indicating higher energetic status/ metabolic efficiency						
	Gut microbiota	Intestine								
	Lysozyme (Units mL ⁻¹)	Plasma	Evaluate the innate immune response	Low levels indicating immunodeficiency						
	Peroxidase (Units mL ⁻¹)	Plasma	Evaluate the oxidative response against pathogens	High levels indicating higher anti-oxidant / immune activity						
	OXY (mM HOCl neutralized)	Plasma	Measure of non-enzymatic defense against ROS	Low levels indicating lower plasmatic anti-oxidant capacity						
	ROMs (mM H ₂ O ₂ equivalents)	Plasma	Measure of reactive oxygen metabolites (pro-oxidant compounds)	High levels indicating higher oxidative damage						
	SOD (Units Mol ⁻¹)	Serum	Measure of enzymatic scavengers of ROS	High levels indicating higher capacity to neutralize free radicals						
	TBARS (µM MDA equivalent)	Serum	Measure of lipid peroxidation	High values indicating higher oxidation of lipid substrates						
	EROD (pmol min ⁻¹ mg protein ⁻¹)	Liver	Biomarker of exposure to environmental contaminants	High levels indicating higher activity of CYT P450						
Telomere length (kb)	Fin	Proxy of lifespan	Short telomere length associate with decreased lifespan							
<i>Individual traits</i>	Nitrogen isotopes ($\delta^{15}N$, ‰)	Muscle	Proxy of the trophic position	-						
	Age (year)	Scale	-	-						
	Total length (<i>LT</i> , cm)	-	-	-						
	Weight (<i>W</i> , g)	-	-	-						
	Presence of parasites	-	-	-						
<i>Chemicals</i>	Organic pollutants (ng/g ⁻¹ dwt)	M, L, S, P*	Quantify the pollutant load	-						
	Molecular weight	-	-	-						
	Log <i>K_{ow}</i>	-	Tendency of a compound to move from the aqueous phase into lipids	Larger values indicating more hydrophobic molecule						
	Log <i>k_M</i>	-	Metabolic transformation rate	Larger and positive values indicating higher biotransformation rate						

Toxicological relevance

Ecological relevance

CHAPTER III.

Effects of individual traits on the pollutant load and biomarker responses

*« Hélas ! ai-je pensé, malgré ce grand nom d'Hommes,
Que j'ai honte de nous, débiles que nous sommes !
Comment on doit quitter la vie et tous ses maux,
C'est vous qui le savez, sublimes animaux !
A voir ce que l'on fut sur terre et ce qu'on laisse
Seul le silence est grand ; tout le reste est faiblesse. »*

La mort du loup, Alfred de VIGNY.

III – Effects of individual traits on the pollutant load and biomarker responses

This chapter essentially summarizes and discusses the results from **Article 2**⁵. The aim was to investigate the effects of the individual traits of chub on their pollutant load, their probability to get infected by *Pomphorhynchus* sp. and on their health status. Specifically, we examined the relationships between levels of several organic contaminants and the following individual traits: *i*) age, *ii*) body size, *iii*) lipid contents and *iv*) trophic position by using a cross-sectional correlational design on wild chubs, naturally infected or not. This publication is attached at the end of the manuscript.

1. Individuals traits and contaminant levels

Many factors have been shown to drive the concentration of organic contaminants in aquatic organisms (Russel et al., 1999; Fisk et al., 2001; Vives et al., 2005; Goutte et al., 2020), from the environmental conditions to the biology (species, trophic level) and physiology (age, sex, and metabolism) of organisms. To get more insight into the transfer of contaminants, we investigated the relationship between individual traits and the body burden in chub tissues, especially in liver and muscle. A high inter-individual variability in body burdens of parent contaminants was observed in our field study, but those variations were not related to length/age (range: 1–9 years, 8.80–41.5 cm), $\delta^{15}\text{N}$ (5.92–12.9‰) or lipid contents (0.56–19.8%), whatever the fish tissue. The magnitude of accumulation usually depends on the hydrophobicity (expressed *via* the octanol-water partition coefficient; $\log K_{ow}$) and the lipid content of the organisms (but see Stow et al., 1997). For hydrophobic substances, such as PCBs and organochlorine pesticides, lipid equivalent concentrations have been reported to increase with increasing trophic position and stable isotope ratios ($\delta^{15}\text{N}$; trophic biomagnification) (Dromard et al., 2018), as well as age or length (accumulation during growth, Vives et al., 2005; Madenjian, et al., 2017; Harried et al., 2020), due to their resistance to chemical and metabolic degradations. However, for metabolizable compounds (phthalates, PAHs and pyrethroids) these relationships are not straightforward (D'Adamo et al., 1997; Mackintosh et al., 2004; Snyder et al., 2019; Struch et al., 2019; Goutte et al., 2020). Recently, Goutte et al., (2020) reported a pronounced trophic dilution of PAHs and phthalates in a freshwater food web, attributed to the general increase in the rate of metabolic transformation with increasing trophic level. Overall,

⁵ Molbert, N.; Alliot, F.; Leroux-Coyau, M.; Médoc, V.; Biard, C.; Meylan, S.; Jacquin, L.; Santos, R.; Goutte A. Potential benefits of acanthocephalan parasites for chub hosts in polluted environments. *Environ. Sci. Technol.* **2020**, *54*, 5540–5549. (doi:10.1021/acs.est.0c00177)

laboratory and field studies indicate that such contaminants (*i.e.*, easily degraded by vertebrates) do not biomagnify in aquatic food webs. However, depending on the chemical properties of the compounds different trends can be observed (Gobas et al., 2003; Mackintosh et al., 2004). For instance, high molecular weight phthalate esters (*e.g.*, DEHP, DnOP) show evidence of trophic dilution in aquatic food webs, for which metabolic transformation is a key mitigating factor. On the other hand, low and intermediate molecular weight phthalates (*e.g.*, DMP, DEP, DiBP, DnBP and BBP) did not exhibit significant trends with trophic position or stable nitrogen isotope ratios ($\delta^{15}\text{N}$; Mackintosh et al., 2004). In the present work, using sum concentrations (Σ) could have limited the observation of significant relationships.

One of the originalities of this work was to investigate some of the metabolites of organic contaminants. Given that phthalates and PAHs are subjected to processes that contribute to their rapid elimination in fish, monitoring metabolites of contaminants could help to better assess their exposure to environmental contamination. As mentioned in **Chapter II**, levels of metabolites were comparable to those of parent compounds, which is consistent with recent work (Carré et al., 2020), although their concentrations may be negligible or of the same order of magnitude as their parent molecules depending on the species (Fossi et al., 2012) and tissues analyzed (Yue et al., 2020). In addition, since PAH and phthalate metabolites also have deleterious effects, sometimes being even more noxious than the corresponding parent molecule (Wang et al., 2009; Ferguson et al., 2011), they might be of vital importance in ecotoxicological studies (see **Chapter V**). In this thesis, the level of both phthalate and PAH metabolites in chub liver significantly decreased with total length and age (Figure 3.1).

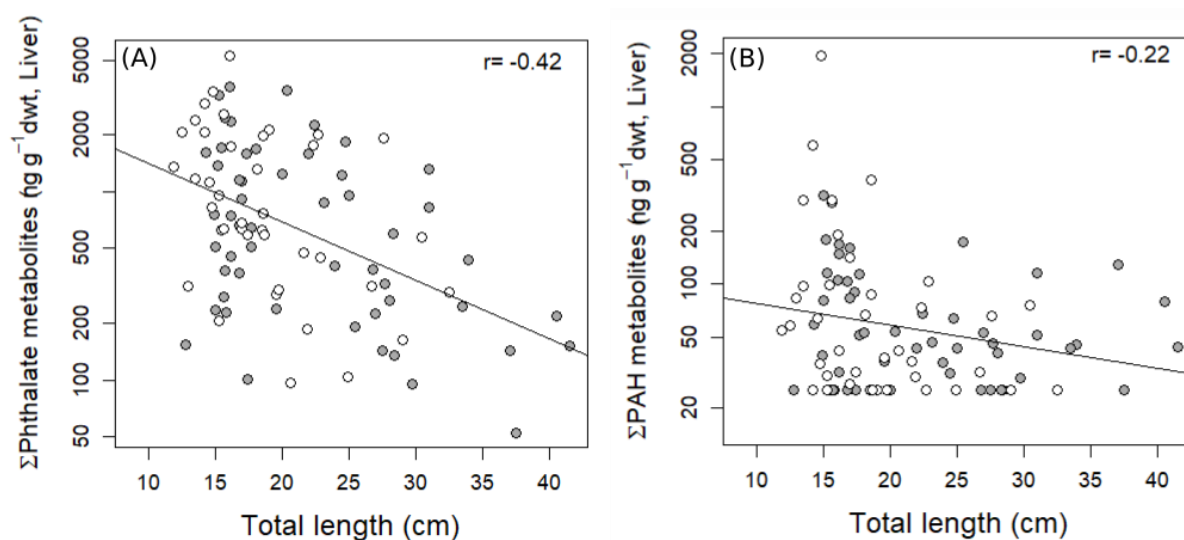


Figure 3.1. Relationship between metabolites of organic contaminants ([A] phthalate metabolites and [B] PAH metabolites) and the body size of chub, infected (grey) or not (white) by *Pomphorhynchus* sp., under natural settings in 2016.

Overall, studies of phthalate metabolites levels in wildlife are rare (Fossi et al., 2012, 2014; Valton et al., 2014; Ros et al., 2015, 2016; Brock et al., 2016; Fourgous et al., 2016; Bainsi et al., 2017; Goutte et al., 2020; Rian et al., 2020; Yue et al., 2020). In addition, authors rarely explore the relationship between body length and phthalate metabolite levels in wild organisms, although this could allow us to assess the bioaccumulation of these toxic substances within organisms. Recently, two studies reported no significant correlations between phthalate monoesters and alligator or porpoise length (Brock et al., 2016; Rian et al., 2020). Our work is, to our knowledge, the second to investigate such a relationship in fish (Fourgous et al., 2016). Therefore, little comparisons with previous studies are possible and our results can only suggest differential exposure to contaminants throughout life or a variation in their efficiency to detoxify organic compounds with age. Still, the degree to which aging affects hepatic metabolism in fish is not known, and should therefore be further investigated. Alternatively, since this study is transversal and not longitudinal (*i.e.*, no repeated analyses of the same individuals during its lifespan), we could not exclude the “selection hypothesis” stating that infected fish bearing a high pollutant load are progressively eliminated from the population. Although PAH metabolites have received more attention, in regard to phthalate metabolites, the reported relationships with body length are somewhat contradictory (Vuorinen et al., 2006; Baali et al., 2016). In eelpout, *Zoarces viviparus*, biliary concentrations were significantly and positively correlated with body length and weight, whereas in other fishes no such correlations were not observed. In this thesis, sex was not recorded although sex-specific physiological mechanisms (*i.e.*, metabolic and elimination pathways) might affect pollutant bioaccumulation (Madenjian, et al., 2017). Indeed, sex-specific differences in biliary PAH metabolite concentrations were previously observed, with males typically characterized by higher contaminant burdens (Tuvikene, 1995; Vuorinen et al., 2006), thought to be linked to sex hormones. The cytochrome P450 mixed function oxidase (MFO) enzymes play a major role in the metabolism of important endogenous substrates as well as in the biotransformation of xenobiotics, including PAHs. In fact, estrogens are known to inhibit the MFO system (Stegeman et al., 1982) and in rainbow trout, *Oncorhynchus mykiss*, males EROD activity has been recorded to be higher than in females (Förlin & Haux, 1990).

2. Individuals traits and parasite infection

Infection probability did not vary with fish age but it was positively influenced by body length. This is likely due to the extensive opportunity for exposure to the parasite (Mille et al., 2020),

through a higher probability to consume infected preys (*e.g.*, gammarids). While the trophic position is an important factor in tracking the fate of contaminants in the environment, carbon and nitrogen stable isotopes have been largely used in trophic ecology, as predators' isotopic ratios are directly linked with those of their diet. Here the use of stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) revealed that the trophic niche of infected chubs, measured as the standard ellipse area (*i.e.*, the isotopic niche), showed different patterns depending on the sampling sites (Figure 3.2). With the exception of fish populations from Bry-sur-Marne and Frignicourt (not enough uninfected fish to draw the ellipse), trophic niches of parasitized chubs were consistently smaller compared with uninfected conspecifics. For half of the populations under study, these niches of infected chubs sat within that of uninfected ones (*i.e.*, niche constriction), and the other showed partial niche divergence. This result suggests a potential trophic specialization, with infected fish consuming specific food items that are also within the dietary range of uninfected chubs. The role of parasite infection in trophic niche variation has been well documented in the literature (Britton & Andreou, 2016; Pegg et al., 2017). For example, tapeworm causes infected sticklebacks, *Gasterosteus aculeatus*, to eat smaller preys (Milinski, 1984), or selectively preyed on larger items than uninfected individuals, which compensated for the energy cost of infection (Ranta, 1995). Despite this variation in trophic niche, and potentially in food items, infected chubs appeared to maintain their energetic requirements. Indeed, infected and uninfected chubs did not differ in their body condition (Fulton's condition index; see Chapter V). Even though it was not observed in this thesis, this non-lethal consequence of parasite infection could drive different feeding behavior (Milinski, 1984; Ranta, 1995), thereby leading to differential exposure to contaminants for the host (see Chapter IV). Alternatively, dietary specialization could be a driver of *Pomphorhynchus* sp. infection rather than a consequence (Britton & Andreou, 2016), given that longer (older) fish had higher probability of being infected and that diet composition varies with fish body size and/or age. Recently, Pegg et al., (2017) reported a consistent niche specialization in different fish host populations infected by a copepod gill parasite. They speculated that infected fish increased their predation on preys requiring relatively low-energy expenditure to capture (*i.e.*, abundant and/ or relatively slow moving) as a consequence of parasite-associated energetic costs. Further investigations conducted on larger sample size could help distinguished potential effects by dividing infected and uninfected fish into subgroups according to their length or age.

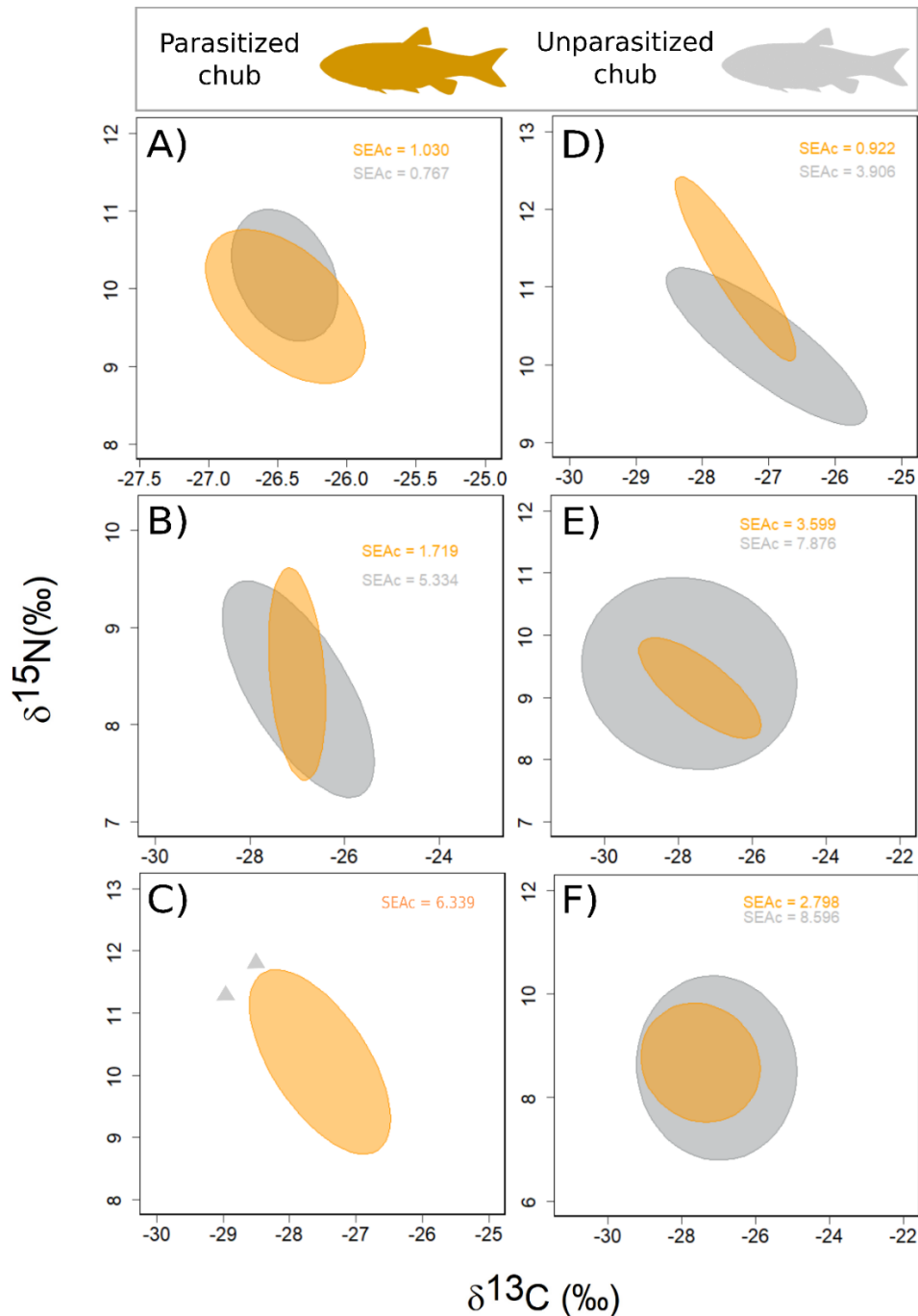


Figure 3.2. Stable isotope ellipses depict trophic niche breadth and overlap between infected and uninfected chubs among the sampling locations on the Marne River and its tributaries collected in 2016; (A) Bry-sur-Marne, (B) Celles-les-Condés, (C) Frignicourt, (D) Torcy, (E) Ferté-sous-Jourarre and (F) Verneuil; based on SEAc analysis. For (F), only two uninfected fish were captured, preventing quantification of niche metrics.

3. Individuals traits and biomarker responses

In fish, variations in the stress response are difficult to predict and they often vary according to class of chemicals (see **Chapter IV**), species sensitivity (Trenzado et al., 2006) and several

biological factors (Winston & Di Giulio, 1991). In this section, we discuss the influence of individual traits on biomarker responses.

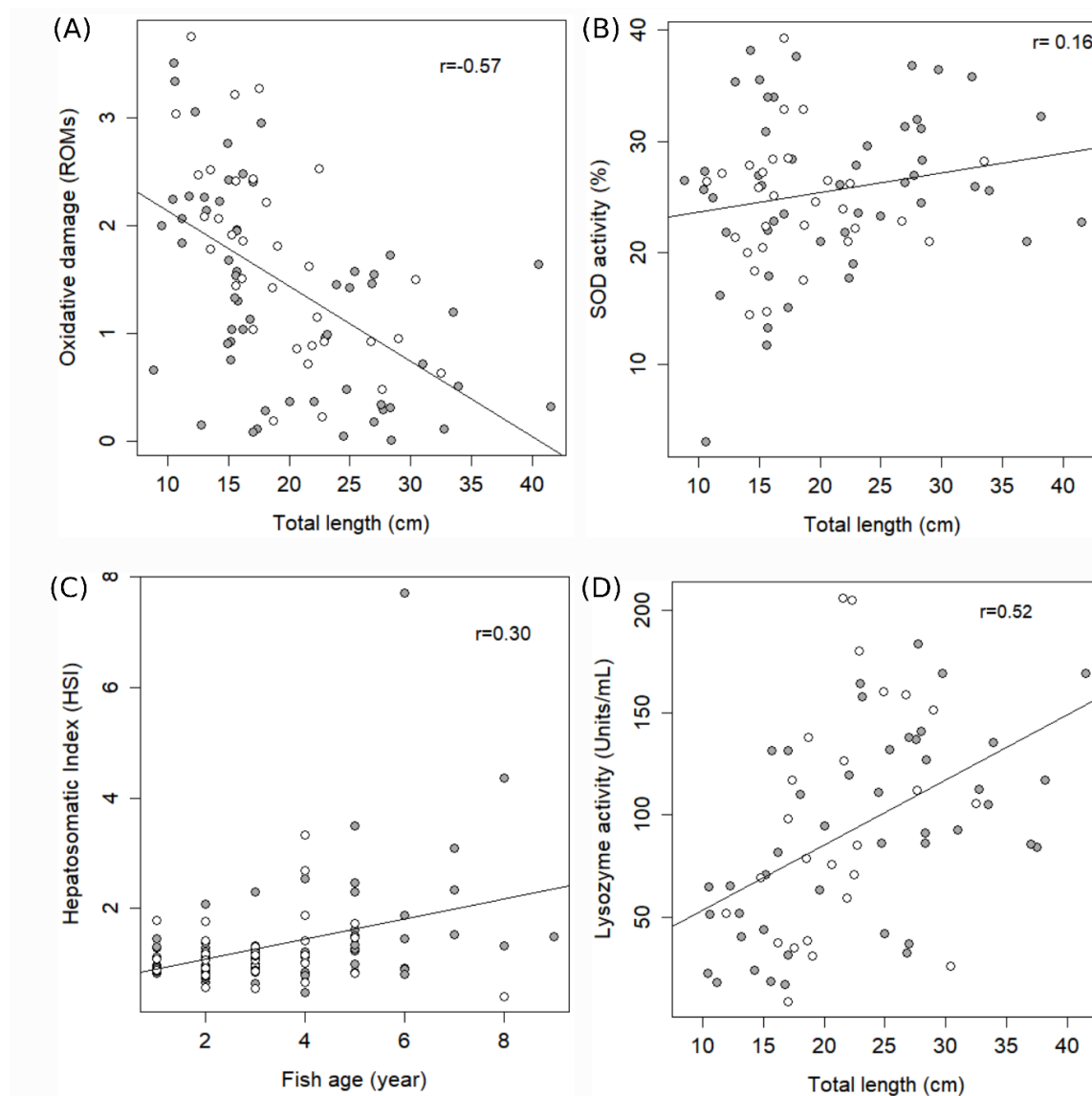


Figure 3.3. Relationship between biomarkers and age/body size of chub, according to their infection status (grey: infected and white: uninfected), under natural settings in 2016. (A) Oxidative damage (ROMs; mM H₂O₂ equivalents), (B) superoxide dismutase activity (SOD; %), (C) hepatosomatic index (HSI) and (D) lysozyme activity (Units/mL).

Interestingly, oxidative damage (ROM concentrations) negatively correlated with body size, whereas enzymatic antioxidant activity (SOD) significantly increased with body size (LMM: $F_{(1,74.2)}=6.322, p=0.014$; Figure 3.3A and 3.3B). This suggests that longer fish (or older, as body size and age were highly correlated; $F_{(1,96)}=435.5, p<0.001, r=0.86$) could limit oxidative damage through higher capacity of the enzymatically based antioxidant system. There are few studies on the effect of age (or length) on enzymatic antioxidant activities in wild fish. In general, the defense mechanisms that protect fish against oxidative stress weaken with age

(Martínez-Álvarez et al., 2005), resulting in a gradual imbalance in the oxidative status (in favor of the ROS) with potential functional deteriorations. In line with our results, Sanz et al. (2001) looked at the activities of SOD in the first life phases (at 0, 1, 2, 3 and 5+ years) of the sturgeon, *Acipenser naccarii*, and reported an increase in SOD, as well as other antioxidants, in red blood cells and plasma with age. This enhancement in antioxidant responses with age is also consistent with the liver hypertrophy in older fish (as measured by the hepatosomatic index, HIS) (Figure 3.3C), which has been explained in previous works by a higher metabolic activity of fish (Williams & Iatropoulos, 2002; Tenji et al., 2020). Another explanation for our findings is that younger fish tend to have lower energy content stored as fat than mature individuals (Jonsson & Jonsson, 2003; Martin et al., 2017), which may constrain allocation to other functions like antioxidant production when faced with an oxidative stress condition. Recent experimental studies demonstrated a link between body condition, antioxidant defenses (SOD) and ROS levels, reflecting the investment of healthier individuals in antioxidants and reduction of ROS (Clotfelter et al., 2013; Friesen et al., 2019). Collectively these results suggest that the antioxidant system becomes more functional as the organisms reach greater stages of maturity or that investment in endogenous antioxidants, such as SOD, may be condition-dependent. If antioxidant components were indeed costly, body condition (Fulton's condition index) should have been positively correlated with SOD levels, which was not the case in this correlative study ($F_{(1,75)}=0.245$, $p=0.621$). However, we cannot exclude a time-lagging relationships between body condition and SOD production, especially with a cross-sectional correlational design. Finally, lysozyme activity in chubs was positively correlated to body length (Figure 3.3D). Given that significant dietary shifts occur along their growth and that nutrition influence defense mechanisms (Saurabh & Sahoo, 2008), "older" fish might be able to increase or maintain their non-specific humoral immunity. In fact, Studnicka et al. (1986) reported that the highest level of the enzyme occurred in spawners.

CHAPTER IV.

Fate of organic pollutants in host-parasite systems

“Quelle que soit la chose qu'on veut dire, il n'y a qu'un mot pour l'exprimer, qu'un verbe pour l'animer et qu'un adjectif pour la qualifier. »

Pierre et Jean, Guy de Maupassant.

IV – Fate of organic pollutants in host-parasite systems

This chapter essentially summarizes and discusses the main results from **Articles 2 and 4**⁶. The aim was to investigate the distribution of several organic contaminants, and some of their metabolites, in a host-parasite system and to evaluate the effect of *Pomphorhynchus* sp. infection on the pollutant load of its final host by using both correlative and experimental approaches on wild chubs, naturally infected or not. Specifically, we investigated the capacity of intestinal worms to accumulate organic compounds and we tested whether accumulation patterns are explained by physico-chemical properties of the contaminants under study (hydrophobicity, metabolic transformation rate and molecular weight).

1. Bioaccumulation of pollutants in parasite tissues

Of the 118 fish captured in 2016, 73 (61.9%) were infected by *Pomphorhynchus* sp., with a prevalence ranging from 50% to 90%, which did not significantly differ across sampling sites. Similarly, the prevalence of intestinal worms and their intensity of infection did not differ among treatment groups, with 69%, 80% and 67% of infected fish exposed to 0.1X, 1X and 10X, respectively, harboring a mean number (\pm SE) of 3.47 ± 0.32 , 2.54 ± 0.35 and 2.67 ± 0.26 *Pomphorhynchus* sp. This experimental approach confirms that these intestinal worms tolerate very high pollutant burdens in their hosts (see [Sures et al., 2017](#) for review). Intestinal worms had higher accumulation capacities than their final host for most of the contaminants investigated ([Table 4.1](#)). For instance, *Pomphorhynchus* sp. accumulated ~ 2 to 700 times more Σ PAHs than the host, with levels of individual PAH congeners up to 5,000 times higher in parasites than in fish muscle. Bioaccumulation patterns were however more contrasted for persistent pollutants (PCBs, OCPs and PBDEs), with similar or even lower (for OCPs) concentrations in parasite tissues compared to fish matrices. Our results corroborate a previous study, in which *Monobothrioides* sp. parasitizing the Nile tilapia, *Oreochromis niloticus*, accumulated Σ PCBs x4–8 and up to x6–14 for *Proteocephala* sp. compared to the muscle of infected Nile perch, *Lates niloticus* ([Oluoch-Otiego et al., 2016](#)). However, reported lipid-corrected concentrations of PCBs in parasites species (mostly endoparasites such as acanthocephalans) were usually lower than the corresponding concentrations in their hosts ([Persson et al., 2007](#); [Brázová et al., 2012](#)). The literature indeed suggests a fractionation of

⁶ **Molbert**, N.; Millot, A.; Decencière, B.; Collin, Y.; Leroux-Coyau, M.; Alliot, F.; Berthe, T.; Petit, F.; Goutte, A. (*Submitted*) Parasitism reduces oxidative stress and alters gut microbiota of fish host experimentally exposed to PAHs.

organic compounds between parasites and their hosts related to the commonly observed bioaccumulation pattern of organic chemicals, as persistent pollutants have higher tendency to partition in lipids than in the aqueous phase (Arnot & Gobas, 2004). Since parasites generally have lower lipid contents than their hosts, the accumulation level of lipophilic substances is expected to be lower in parasites than in the host tissues. In this PhD work however, the lipid correction was not applied for the following reasons: (i) levels of contaminants did not vary in direct proportion to lipid content (Hebert & Keenleyside, 1995) and (ii) there were no significant differences in lipid content between intestinal worm tissues (Mean \pm SD, min–max ; 4.71 ± 1.37 [2.75–5.81]%) and host matrices (muscle 3.01 ± 1.72 [0.56–8.98]%, liver 6.19 ± 3.99 [1.59–14.5]%, and stomach content 8.76 ± 4.77 [3.35–19.8]%).

Table 4.1. Ratios of pollutant concentrations in *Pomphorhynchus* sp. relative to that in host muscle (n = 19), liver (n = 9) and stomach content (n = 12) and the pollutant load (ng g⁻¹ of dry weight) in parasite tissues (n = 19). * indicates that the ratio (r) significantly differs from 1. (*: $p < 0.05$; **: $p < 0.01$; *** $p < 0.001$)

Compounds	$C_{[\text{Parasite}]} / C_{[\text{chub Muscle}]}$	$C_{[\text{Parasite}]} / C_{[\text{chub Liver}]}$	$C_{[\text{Parasite}]} / C_{[\text{chub Stomach}]}$
	Mean \pm SD	Mean \pm SD	Mean \pm SD
$\Sigma 7$ Phthalates	22.8 ± 32.2 (***)	8.45 ± 7.73 (*)	7.71 ± 12.7 (*)
$\Sigma 16$ PAHs	211 ± 187 (***)	86.2 ± 124 (**)	3.40 ± 1.50 (***)
$\Sigma 7$ 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
$\Sigma 4$ OCPs	6.60 ± 7.46 (***)	2.16 ± 1.00 (**)	0.43 ± 0.52 (*)
$\Sigma 7$ PCBs	3.05 ± 1.86 (***)	1.98 ± 1.48 (*)	1.15 ± 0.77
$\Sigma 6$ PBDEs	3.39 ± 2.44 (***)	0.76 ± 0.53	1.03 ± 0.86

While the accumulation of persistent pollutants was higher in parasite tissues relative to its host ($C_{[\text{Parasite}]} / C_{[\text{Chub Muscle}]}$), the ratio decreased with increasing host weight or length (Figures 4.1A, 1B and 1C), likely because of their bioaccumulation potential in fish during growth (Vives et al., 2005) due to their high lipophilicity ($\log K_{ow} > 6$) and resistance to degradation. This highlights the importance of studying the physico-chemical properties of chemicals to assess the underlying factors that play a role in their distribution patterns in organisms. In previous studies, the variability in the bioaccumulation ratio could not be explained by either their hydrophobicity or molecular size (Persson et al., 2007; Brázová et al., 2012). A major drawback was that they tended to focus on a single family of organic compounds resulting on a narrow range of chemical properties (e.g., $\log K_{ow}$: [5.6–7.4]). The originality of this PhD work is to

investigate a wide range of currently-released pollutants and their metabolites in a common freshwater fish species (*e.g.*, $\log K_{ow}$: [1.8–8.2]).

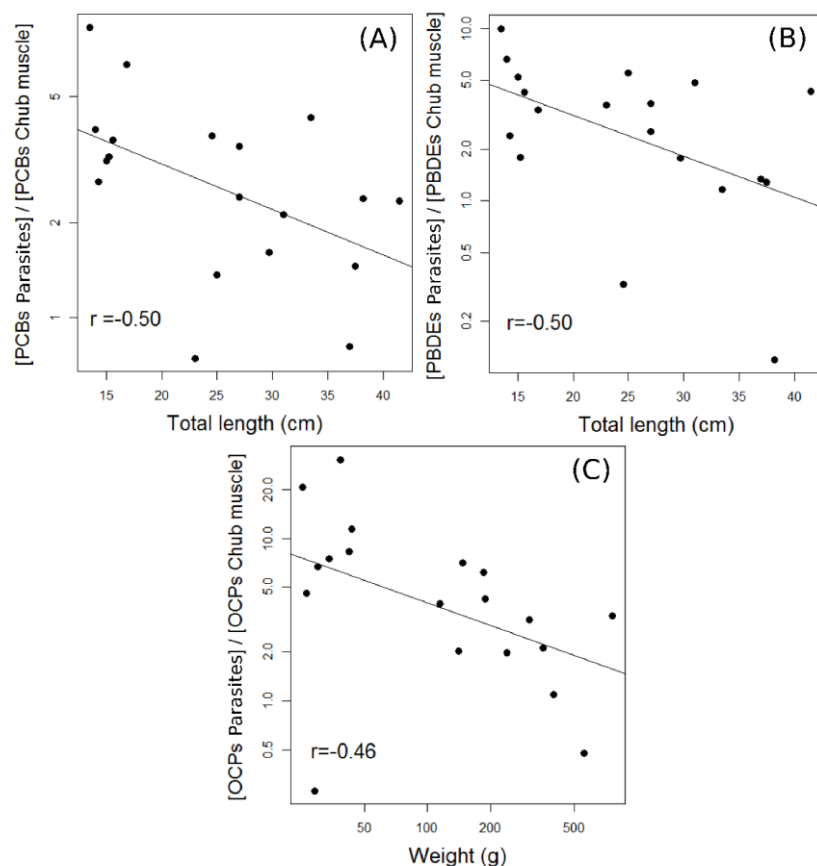


Figure 4.1. Ratios of pollutant concentrations (ng g^{-1} of dry wt) in *Pomphorhynchus* sp. relative to that in host muscle ($n = 19$) for (A) PCBs, (B) PBDEs and (C) OCPs in relation to fish length (g) and weight (cm), respectively.

Interestingly, metabolizable pollutants, such as PAHs and phthalates, had the highest accumulation ratios in parasites. Up to now we had very little knowledge on the mechanistic explanation of high bioaccumulation of organic contaminants in parasite tissues. However, we found that accumulation capacities of acanthocephalan parasites ($C_{[\text{Parasite}]} / C_{[\text{Chub Muscle}]}$) increased with the metabolic transformation rate ($\log k_M$) of the molecule (Figure 4.2B), but were unrelated to other physico-chemical parameters (molecular weight, hydrophobicity; Figures 4.2A and 2C) and biological factors (lipid contents, trophic position). One may predict that acanthocephalan parasites are less able to degrade and excrete organic 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 (Livingstone, 1998; Ruus et al., 2001). More recent data from the literature show indeed that *Pomphorhynchus laevis* had similar activity of glutathione peroxidase and glutathione-*S*-transferase than in barbel, *Barbus barbus*, intestine, but were lower than those in the liver (Radovanović et al.,

2015). Specifically, our study reveals that molecules with $\log k_M > -1$ accumulated preferentially in parasites, therefore acting as a sinkhole of organic contaminants, suggesting a key role of metabolic processes in the differential distribution of organic contaminants within the host-parasite system.

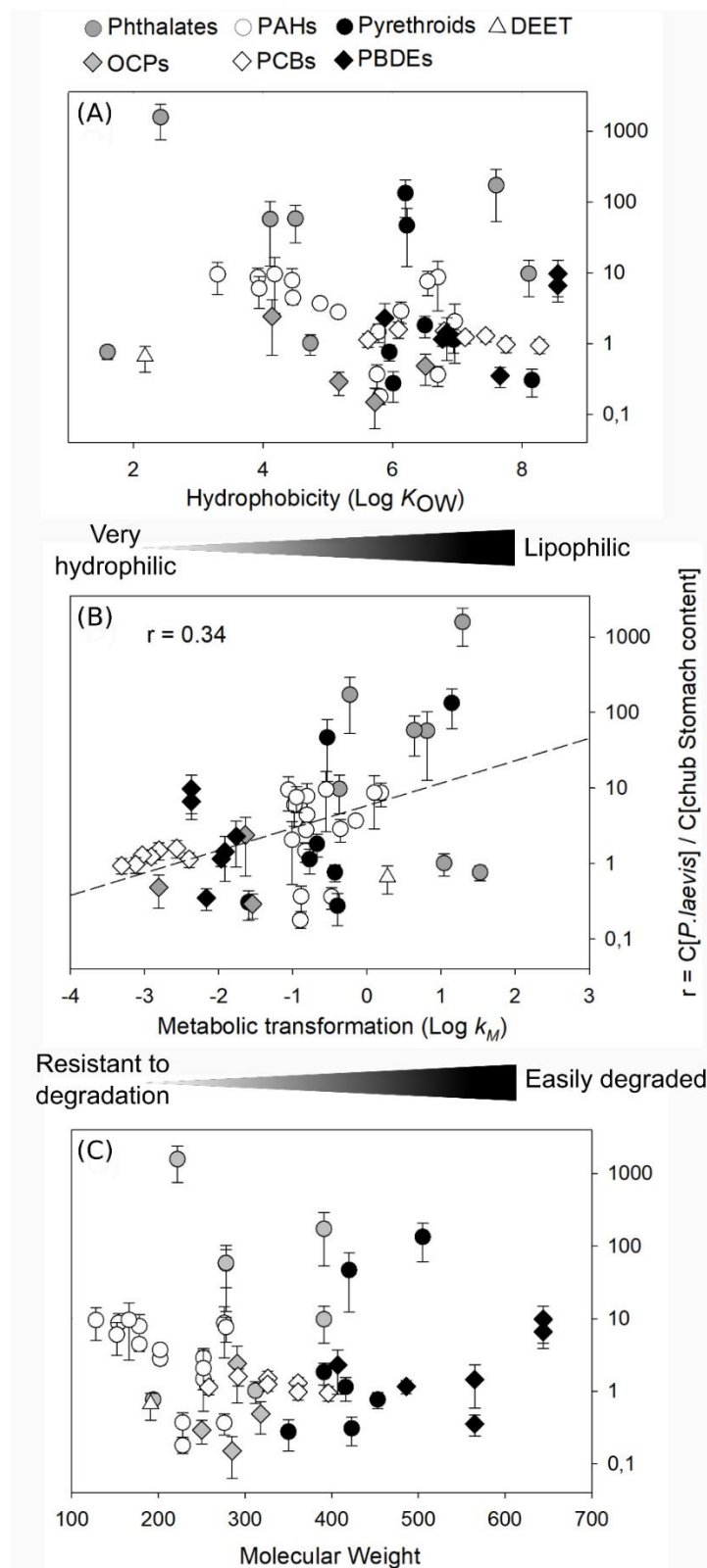


Figure 4.2. Bioaccumulation ratio (r ; mean \pm SD) of 48 organic pollutants in *Pomphorhynchus* sp. relative to fish stomach content in relation to (A) their hydrophobicity ($\log K_{ow}$), (B) metabolic biotransformation ($\log k_M$) and (C) molecular weight.

Because of their lack of digestive tract and basic synthesis pathways, acanthocephalan parasites take up substances (*e.g.*, lipids) directly from the host intestine (Köhler & Voigt, 1988). Consequently, worms living in the host's intestine have the same food as the host, supplemented by some molecules originating from the host (Aitzetmüller *et al.*, 1994). It is thus likely that pollutants in acanthocephalan tissues are taken up by the worms concurrently with nutrients or other substances. Indeed, contamination profiles of organic contaminants were highly similar, especially between the host's digestive tract and parasites tissues, excluding pyrethroid pesticides, with $\geq 70\%$ of the pollutant load dominated by PAHs and plasticizers (Figure 4.3). Interestingly, a greater proportion of PAHs was measured in the digestive tract (site of attachment of adult worms) compared to other fish matrices and PAHs had the highest accumulation ratios (levels of contaminants in *Pomphorhynchus* sp. relative to that in its host).

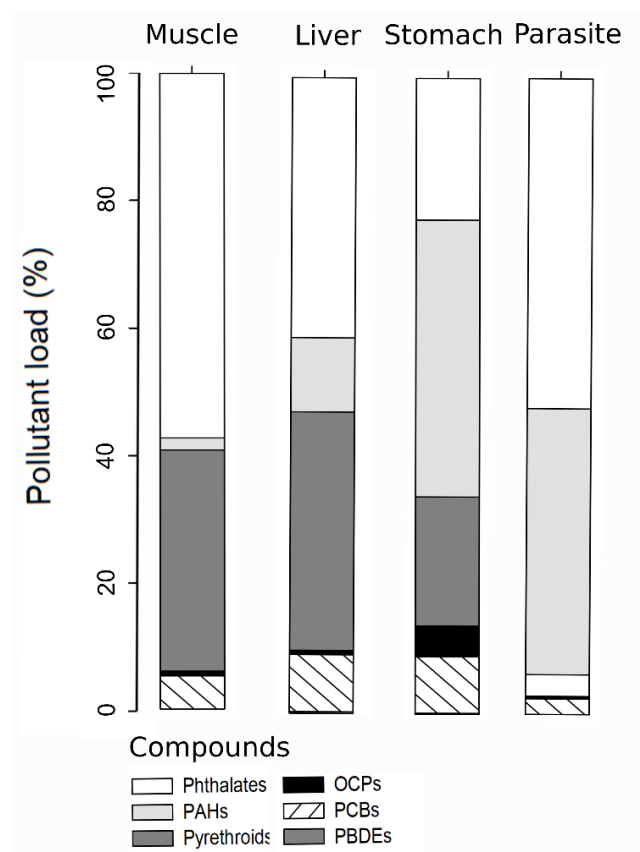


Figure 4.3. Contamination profile of organic contaminants in chub matrices (muscle [$n = 19$], liver [$n = 9$] and stomach content [$n = 12$]) and parasite tissues ($n = 19$).

The very low level of pyrethroid pesticides in parasites could not be explained by the chemical properties. Pyrethroid pesticides were the second most important compounds detected in fish tissues, even though they contributed less significantly to the total contamination in stomach content (Figure 4.3), and share similar chemical properties to PAH and phthalate compounds, which accumulated in parasites tissues. Certainly, more intensive laboratory work is necessary to define the behavior of chemical fractionation of pyrethroid pesticides in host–parasite systems. Together, the composition profiles and the physico-chemical properties shed some light on the exposure pathways of organic contaminants and the bioaccumulation processes in the host-parasite system under study (Figure 4.4).

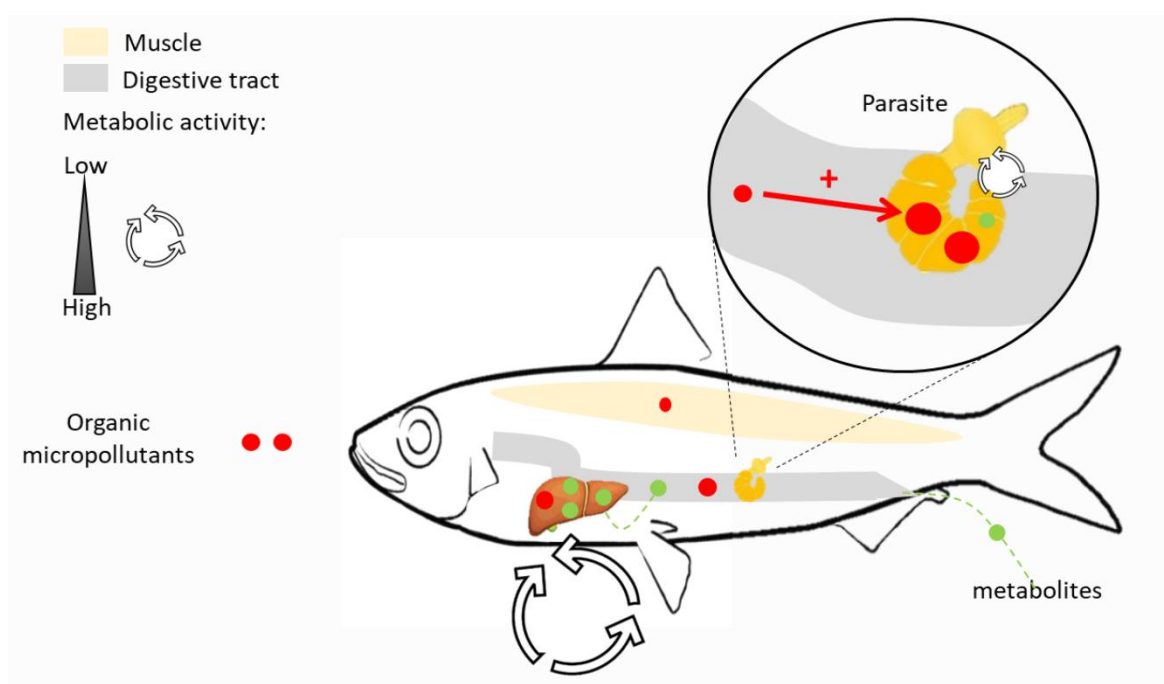


Figure 4.4. Suggested mechanisms explaining the differences in the pollutant load between parasite tissues and various matrices of wild chub, *Squalius cephalus*.

2. Effect of parasites on the host pollutant burden

Contrary to our expectations and despite the preferential accumulation of pollutants in acanthocephalans compared to their host, infected chubs were not significantly less contaminated than uninfected ones, whether we used a correlative or experimental approach (Figures 4.5 and 4.6). Former studies however evidenced the capacity of intestinal parasites to reduce the pollutant load of their fish host (Vidal-Martínez et al., 2003; Brázová et al., 2012).

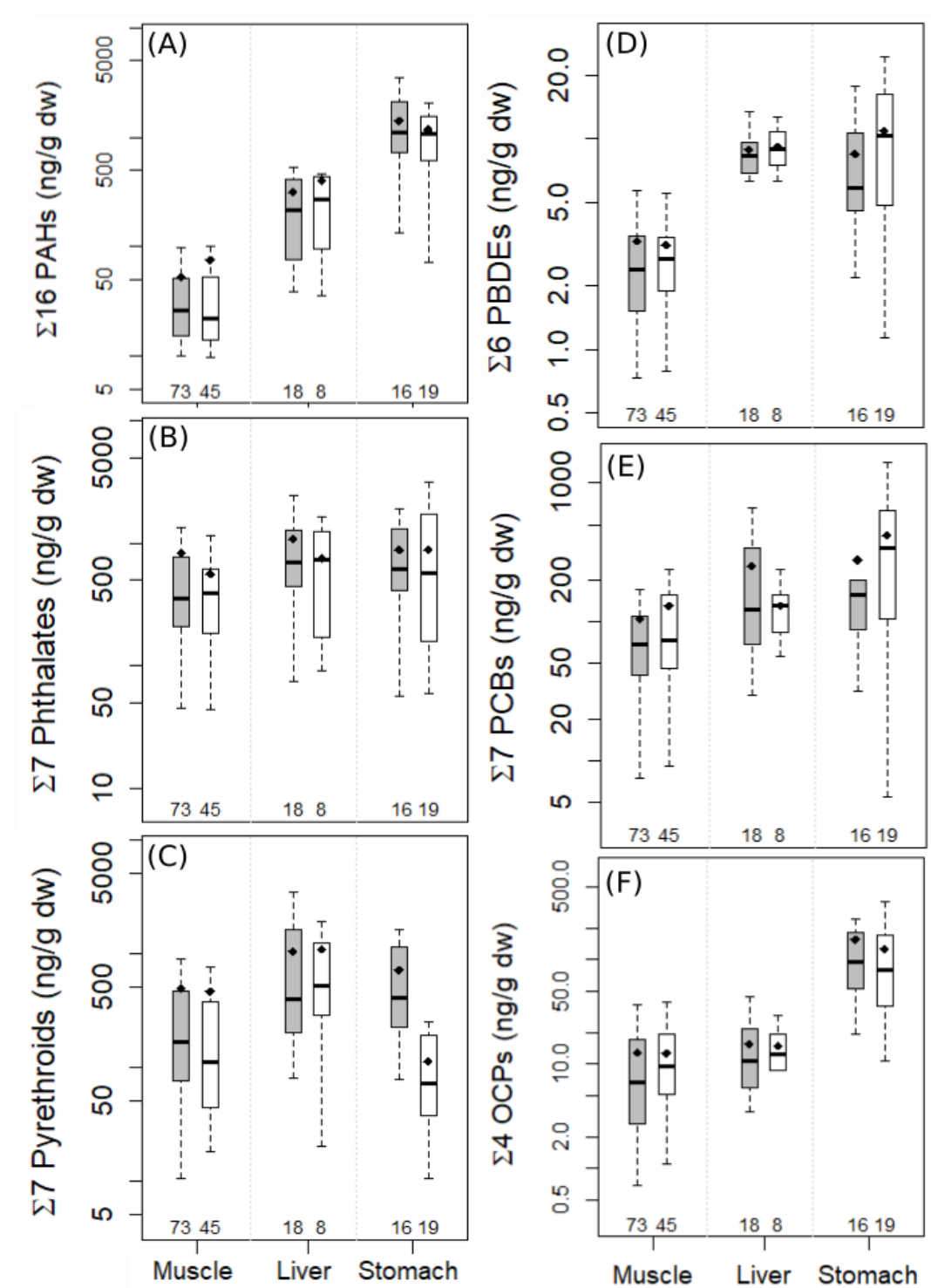


Figure 4.5. Pollutant load ([A-C] metabolizable and [D-F] persistent chemicals) in the muscle, liver and stomach content of infected (grey) and uninfected (white) chub, under natural settings in 2016. Numbers below boxplots refer to the sample size. Filled diamonds represent the arithmetic mean.

Brázová et al. (2012) found that the acanthocephalan *Acanthocephalus lucii* attached to the intestine of perch, *Perca fluviatilis*, accumulated significantly higher levels of PCBs than the

muscle, liver, kidney, brain, and adipose tissue of their host. In infected perch, PCB levels in the liver and muscle were about 20 times lower and 3 times lower compared to uninfected fish, respectively. Similarly, the Mayan catfish, *Ariopsis assimilis*, parasitized by the larval digenean *Mesostephanus appendiculatoides* had half as much DDT concentrations as parasite-free ones (Vidal-Martinez et al., 2003).

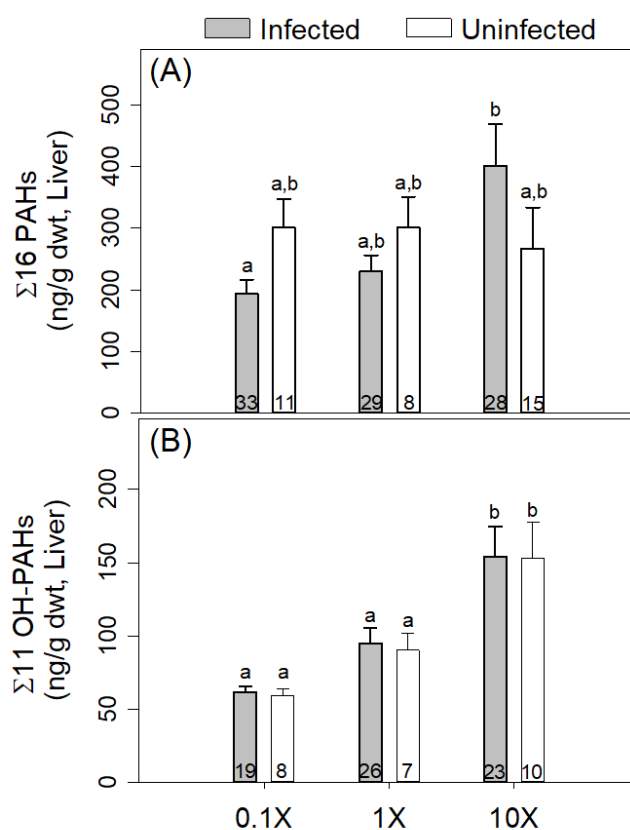


Figure 4.6. Levels of (A) Σ 16 PAHs and (B) Σ 11 OH-PAHs in chub (ng g^{-1} dry weight, Liver) among treatment group (levels of PAH exposure). Numbers represent sample size. Significant differences (*post-hoc tests*: $p < 0.05$) between groups are indicated by different letters [a,b]. Data are expressed as the mean \pm standard error.

A potential explanation is the weak parasitic load encountered in 2016 (median [range] of parasite biomass per fish, 14.8 [0.99–500] mg of dwt) and 2019 (27 [1–143] mg of wwt), probably lessening the detoxification process of infected fish. Moreover, environmental conditions, especially pollution, may positively or negatively influence host–parasite interactions by increasing parasite abundance and diversity in polluted waters (Poulin, 1992), or by compromising the survival of intermediate hosts (*e.g.*, gammarids, Gerhardt et al., 2011) and the life cycle of parasites. Carreras-Aubets et al. (2012) showed that both directly

transmitted ectoparasites and endoparasites with complex life-cycles, transmitted *via* food chains, exhibited a decrease in abundance and prevalence with the increase in PCB levels. In this thesis, the prevalence of *Pomphorhynchus sp.* in fish did not differ among sampling sites despite the high variation of organic pollution in water and sediments (*e.g.*, Σ PCBs: 2.04–57.5 ng g⁻¹ dwt, data not shown), but the abundance of infected gammarids was not monitored. Although infected and uninfected hosts did not differ in their pollutant load, parasite intensity had a marginal effect on the levels of PAHs (Linear Mixed Models, Lot x Parasite intensity: $F_{(1,105)}=2.293$, $p=0.064$; **Figure 4.7A**) with the most parasitized fish (> 4 parasites) showing lower contaminant body burden compared to uninfected ones at the lowest experimental exposures to PAHs (0.1X and 1X). In addition, the pollutant load in infected chub decreased slightly with increasing parasite biomass (**Figure 4.7B**). This suggest that higher numbers of *Pomphorhynchus sp.* could have interfered with the absorption of parent organic compounds by the host. **Sures (2002)** showed that *in natura*, acanthocephalan parasites affected trace metal concentrations in the liver of perch as the levels of several minerals were negatively correlated with the size of the infrapopulation.

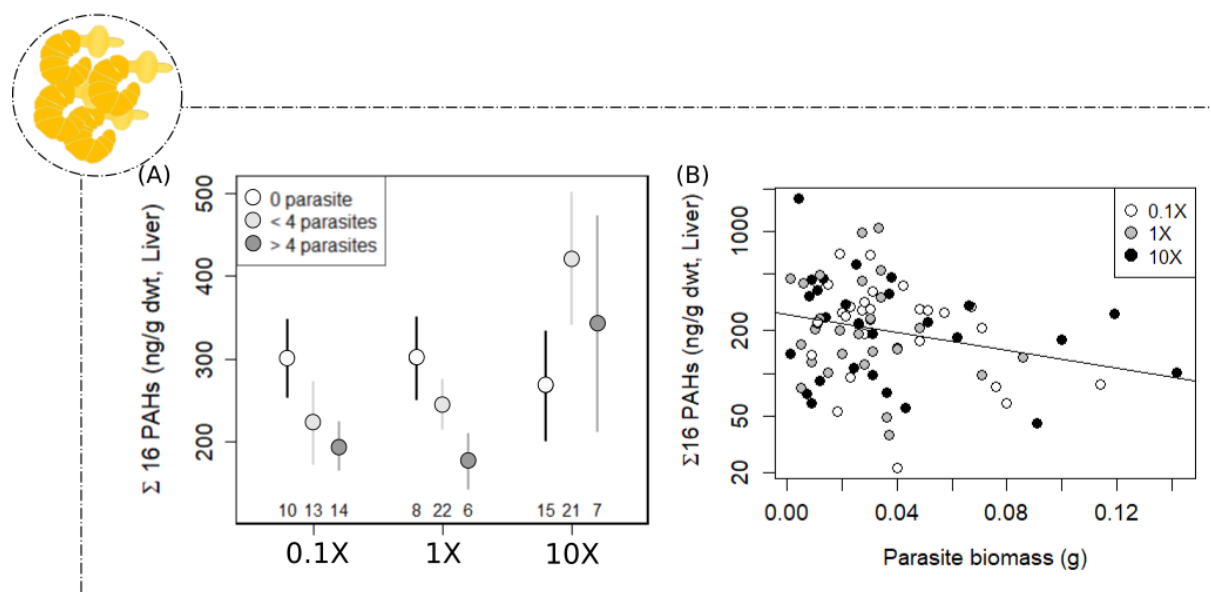


Figure 4.7. Effect of the **(A)** infection intensity of *Pomphorhynchus sp.* and **(B)** parasite biomass (as measured by the wet weight of the infrapopulation of *Pomphorhynchus sp.*) on the level of PAHs in chub liver, after five weeks experimental exposure to PAHs in 2019 (0.1X, 1X and 10X of PAHs).

In line with our findings on parent contaminants, metabolite levels in the liver did not differ significantly between infected and uninfected fish (**Figures 4.6B** and **4.8**) and were not statistically different from the levels in parasite tissues. This is the second work to investigate

the fate of organic pollutants in a host-parasite system. Recently, [Soler-Jiménez et al. \(2020\)](#) reported markedly higher PAH metabolite concentrations in parasites, at least four orders of magnitude higher, than those in their fish hosts. While parasites did not accumulate higher levels of metabolites in our study, the detection of by-products in the intestinal worm highlights either that *Pomphorhynchus* sp. are able to metabolize certain chemicals ([Spann et al., 2015](#); [Henríquez-Hernández et al., 2017](#)) or that they incorporate metabolites readily biotransformed inside host tissues. Although reduced pollutant levels in chubs due to parasitism was not observed under natural condition, a reduction of the amount of accumulated pollutants could lead to a decrease of adverse effects and would be beneficial to the host. Host-parasite interactions could thus have strong implications for ecotoxicological studies. However, this assumption deserves further investigation under controlled conditions.

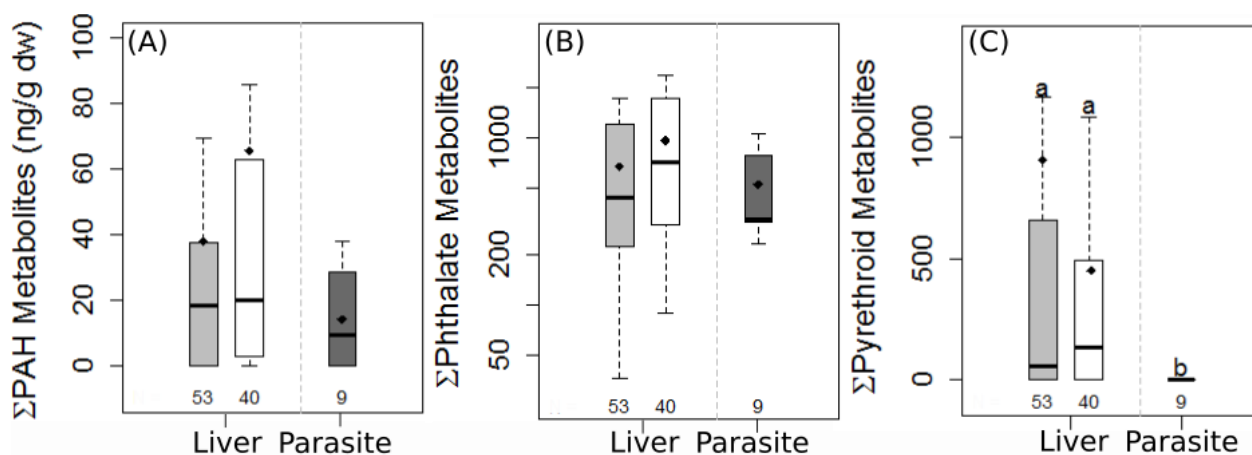


Figure 4.8. Levels of (A) PAH metabolites, (B) phthalate metabolites and (C) pyrethroid metabolites in the liver and of infected (grey) and uninfected (white) chub as well as in parasites tissues, under natural settings in 2016. Numbers below boxplots refer to the sample size. Filled diamonds represent the arithmetic mean. Letters [a,b] indicate significant differences in the pollutant load.

Host responses to chemical pollution and
parasitism

“It is quite conceivable that a naturalist, reflecting on the mutual affinities of organic beings, on their embryological relations, their geographical distribution, geological succession, and other such facts, might come to the conclusion that each species had not been independently created, but had descended, like varieties, from other species.”

The origin of species, Charles Darwin

V – Host responses to chemical pollution and parasitism

This chapter essentially summarizes and discusses the results from **Articles 2, 3⁷** and **4**. Their aim was to investigate the effects of environmental exposure to organic contaminant and infection by *Pomphorhynchus* sp. on the health status of a fish host at different levels of biological organization. Specifically, we examined the relationships between organic contaminant exposure and physiological responses by using a correlative and experimental approach on wild chubs, naturally infected or not. These three publications are attached at the end of the manuscript.

1. At the individual and organ levels

1.1 Body condition and hepatosomatic index

The condition factor (*K*) and hepatosomatic index (HSI) are useful tools to inform on metabolic cost induced by contaminants and indirectly on the energetic status of the exposed individuals. These indices offer relevant information on fish health and can reflect their probability to survive (Robinson et al., 2008). In our field study and experimental approach, neither body condition (Figures 5.1A and 1B) nor hepatosomatic index (Figures 5.1C and 1D) were related to infection status. Importantly, no mortality was recorded over five-week exposure and no external or internal lesions were observed, whether fish hosts were parasitized or not. These results suggest that exposure to environmentally relevant levels of PAHs combined with parasitic infection did not generate higher energetic demands nor compromise the condition of the host, which concurs well with previous studies (Hursky & Pietrock, 2015; Lagrue & Poulin, 2015, Petitjean et al., 2020, **Article 2**). Alternatively, the apparent lack of pathological effects at higher biological scales may be linked to the relatively low abundance (from 1 to 11 per fish in 2019) of *Pomphorhynchus* sp. in the fish host. Intestinal parasite abundance or biomass and their depth of penetration into the host tissue are indeed the main factors known to cause damage to key host organs and alter body condition (Taraschewski, 2000). Interestingly, a recent finding pointed out a quadratic relation between body condition and parasite abundance or biomass, with benefits at intermediate parasite loads for the fish host (Maceda-Veiga et al., 2016). In contrast to parasites, contaminant-related effects were observed on both indices. Liver enlargement was observed in the high exposure group (10X, Figure 5.1D), which usually reflects enzyme induction (*i.e.*, an increase in the rate of hepatic metabolism, as measured by

⁷ Molbert, N.; Angelier, F.; Alliot, F.; Ribout, C.; Goutte, A. (In revision) Fish from urban rivers and with high pollutant levels have shorter telomeres. *Biol Letters*.

the hepatosomatic index), denoting an adaptive response to contaminant exposure (Tenji et al., 2020).

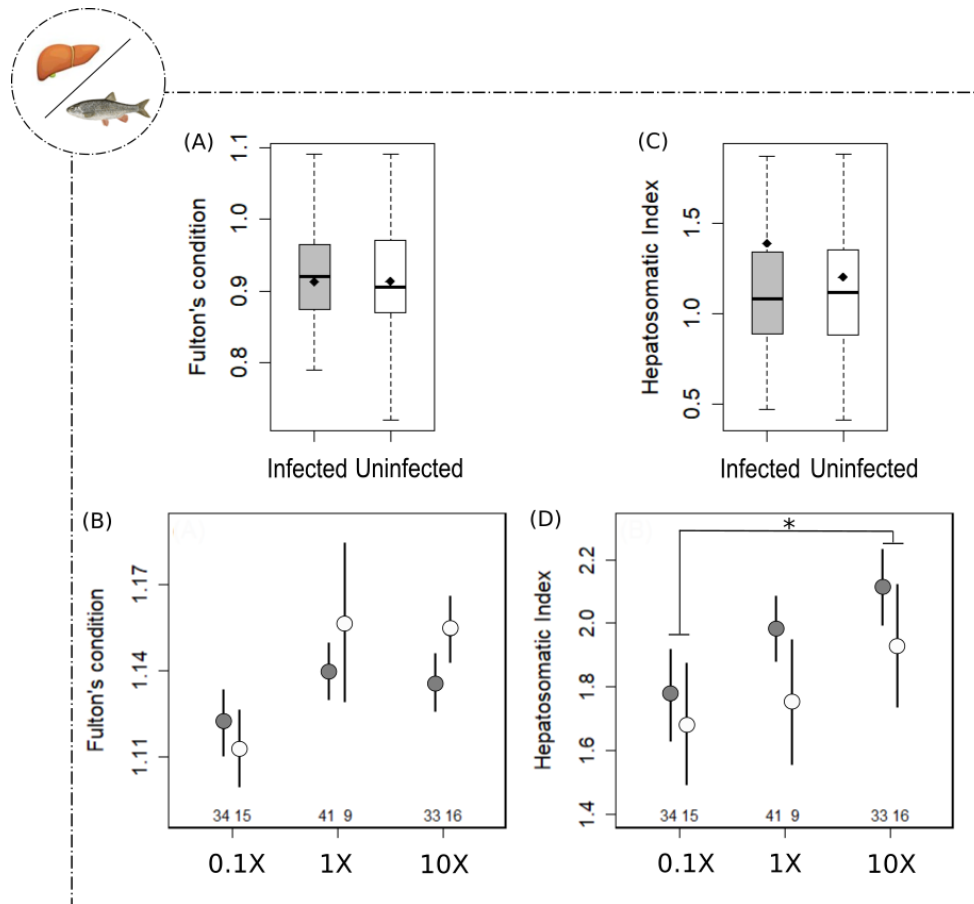


Figure 5.1. Effect of parasite infection (grey: infected, white: uninfected) and pollutant exposure on the body condition and energetic status of chub under natural settings in 2016 (**A**, **B**) and after five-week experimental exposure to PAHs in 2019 (**C**, **D**). Data are expressed as the mean \pm standard deviation for (**A**, **B**) and as the mean \pm standard error for (**C**, **D**). Numbers represent sample size. Filled diamonds represent the arithmetic mean. Asterisks indicate significant effect of treatment (*: $p < 0.05$; **: $p < 0.001$).

This is consistent with the levels of PAHs measured in chub tissues. After five-week chemical exposure, levels of liver metabolites ($\Sigma 11$ OH-PAHs) varied among treatment groups, with significantly higher levels in fish exposed to 10X compared to 0.1X and 1X (Figure 4.6B). In addition, there was a positive correlation between parent PAHs and liver metabolites, which ensures that PAHs-contaminated oil was absorbed and biotransformed after exposure. Accordingly, liver hypertrophy in fish species has been associated with PAH chronic exposure or polluted environments (Everaarts et al., 1993; Larno et al., 2001), suggesting that an increase in the oxidative capacity of this organ may be a common response of fish to some forms of aquatic pollution. Although chemical exposure had no effect on the Fulton's condition at the end of the experiment, this index is 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 (Δ_{T0-T5} Fulton) were negatively affected by increasing PAH exposure (Figure 5.2).

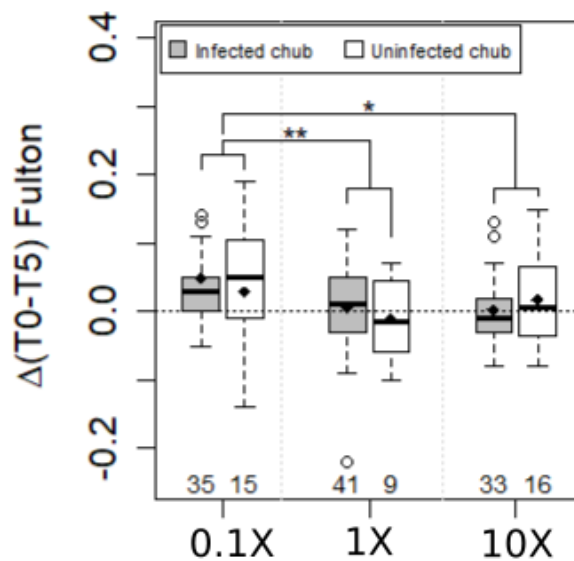


Figure 5.2. Variation of fish host general condition over five weeks of experimental PAH exposure (Δ_{T0-T5} Fulton), among treatment groups (levels of PAH exposure) and for infected (grey) versus uninfected hosts (white). The dotted line indicates no change in body condition. A value below the dotted line means a declining general condition of chubs after 5-week experimental exposure, and vice versa. Filled diamonds represent the arithmetic mean. Numbers below boxplot are sample sizes. Asterisks indicate significant effect of treatment (*: $p < 0.05$; **: $p < 0.001$).

The body condition of chub exposed to 0.1X significantly increased over five weeks compared to 1X and 10X. 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 this thesis. First, fish were hand-fed to monitor feeding activity of each individual and there was no uneaten food left after a feeding. Second, the liver enlargement demonstrates the metabolic efficiency of fish. However, we cannot rule out the possibility that assimilation may have been affected by contaminant exposure. Indeed, changes in the composition of gut microbiota was observed in response to increasing levels of PAHs (see next section). These microbial communities play a critical role in the host's physiology,

especially in energy homeostasis by regulating feeding, digestive and metabolic processes (see [Butt & Volkoff, 2019](#) for review).

1.2 Gut microbiota

In this thesis, we evaluated the impact of both stressors on the diversity and composition of fish gut microbiota, considered as an “extra organ”. Perceived as a valuable physiological marker of stress, the microbiota has attracted increasing attention in ecotoxicological studies ([Evariste et al., 2019](#)), being essential to draw up a health assessment. In the experimental approach, fish contaminated with low (0.1X) or high (10X) levels of PAHs had lower bacterial diversity than T_0 ([Figure 5.3A](#)).

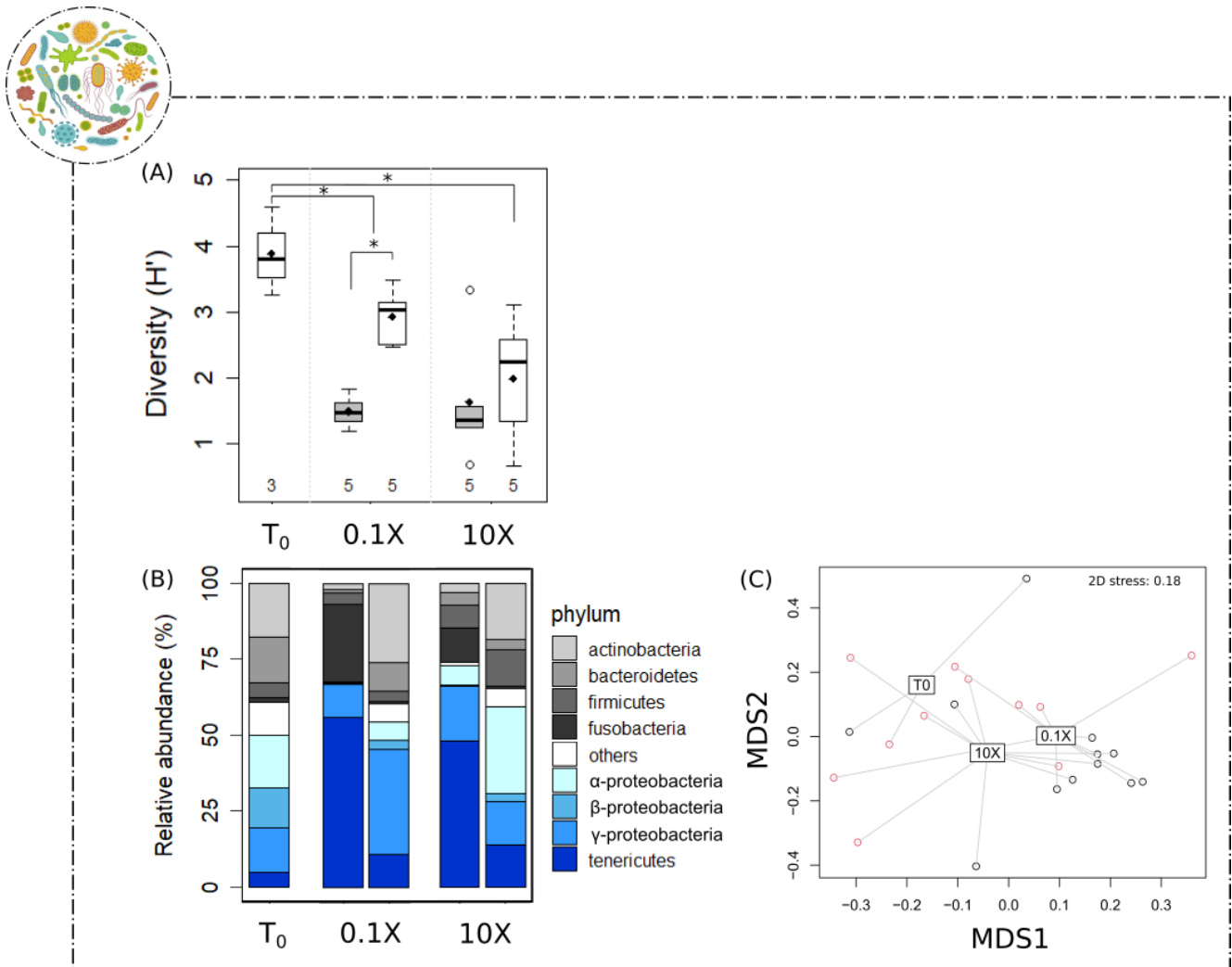


Figure 5.3. Changes in gastrointestinal bacterial diversity and community structure induced by *Pomphorhynchus* sp. infection (grey: infected, white: uninfected) and PAHs contamination. **(A)** Bacterial diversity estimated using the Shannon-Wiener's index (H'). **(B)** Distribution of the main bacterial phyla in fish gastro-intestinal tract. The *Proteobacteria* phylum was detailed for the most abundant proteobacterial classes (α , β and γ). The category 'others' groups minor phyla (<1% of reads) and unclassified reads. Bars represent (from left to right) (T_0): uninfected chubs;

(0.1X): infected and uninfected chubs; (10X): infected and uninfected chubs. (C) NMDS plot of all samples based on Bray-Curtis dissimilarity (infected chub by *Pomphorhynchus* sp. and non-infected ones are colored in red and black tones, respectively). Numbers represent sample size. Filled diamonds represent the arithmetic mean.

This result suggests that fish captivity had an impact on the gut microbiota, with less diverse bacterial communities in chubs at the end of the experiment compared to T₀. This significant difference may reflect the change in environment and diet from natural preys to commercial food. Indeed, it has been shown that diet composition modulates gut microbiota (Bolnick et al., 2014; Michl et al., 2017) which, in turn, shapes intestinal immune responses (Foysal et al., 2019). While PAH exposure had no significant effect on microbial diversity, infected chubs were found to harbour less diverse bacterial communities than non-infected ones (Figure 5.3A). At this point and without data on infected chubs at T₀, it is difficult to determine whether reduced bacterial diversity is a cause or consequence of parasitic infection. It may be assumed that hosts with lower bacterial diversity might be more susceptible to parasite infection (Newbold et al., 2016). Alternatively, parasites that are able to colonize the intestinal tract may impair microbial communities (Ling et al., 2020), through damage to gut epithelium or overlapping resource requirements (Leung et al., 2018). Although negative and positive effects of parasite infection on gut microbiota has been reported in the literature, there is no clear consensus about the direction of the effect (Fu et al., 2019; Ling et al., 2020). In fact, given the complexity of these three-way relationships, results are contingent on the studied systems (Newbold et al., 2016; Midha et al., 2017; Ling et al., 2020). A more diverse gut microbiota is usually associated with « healthy » host, as bacterial communities are more likely to harbour greater gene content or to be more stable in the face of changing environmental conditions (e.g., diet; Rinninella et al., 2019). Although the role of the bacterial community in a set of metabolic functions has been demonstrated, to date no single optimal diversity of gut microbiota, nor its composition, has been reported.

Taxonomic classification of bacterial OTUs (Operational Taxonomic Units) identified 6 main bacterial phyla whose relative abundance was greater than 1% of total reads (i.e., *Proteobacteria*, *Actinobacteria*, *Tenericutes*, *Bacteroidetes*, *Firmicutes* and *Fusobacteria*). In the present work, *Proteobacteria*, *Tenericutes* and *Fusobacteria* were the main phyla detected in the gut of European chubs (Figure 5.3B), which is consistent with bacterial communities reported to predominate in freshwater fish (Michl et al., 2017; Foysal et al 2019; Fu et al., 2019). Based on the OTUs distance matrix, bacterial structural changes were induced by both PAH exposure and parasite infection (Figure 5.3C), with an increase in *Tenericutes* and *Fusobacteria*

abundance and reduction of *Actinobacteria* in infected host, regardless of the level of PAH contamination. The large abundance discrepancy of *Tenericutes* between infected and uninfected fish is exclusively explained by the prevalence of a single genus, *Candidatus bacilloplasma*. Additionally, *C. bacilloplasma*, was more abundant in parasitized fish (55.9 ± 19.9) compared to uninfected ones (10.9 ± 9.95). This genus has been identified in crustacean species, in which it helps hosts to promote digestion process and up-regulate the expression of immune genes (Dong et al., 2018; Foysal et al., 2019). The phylum *Fusobacteria* also brings benefits to the host through the production of butyrate, known to provide energy supply to gastrointestinal cells (Collinder et al., 2003), enhance mucus production and act as an anti-inflammatory (Hamer et al., 2007). Although our results provide a general overview of the taxonomic profile of gut bacterial communities of chub exposed to both contaminants and parasites, we could not conclude on the fitness consequences of the altered gut microbiota. Future research should seek to assess the functional impact of host microbiome composition in the face of environmental pollution and parasite infection, especially given that bacterial communities are able to limit chemicals absorption into the small intestine, biotransform xenobiotics (e.g., PAHs) and regulate the expression of major detoxification enzymes (e.g., CYP450, Collins & Patterson, 2019).

2. At the molecular and cellular levels

2.1 Immunity

The innate immune system of fish is a vital mechanism that constitute the first line of defense against a broad spectrum of pathogens present in the environment and is more important for fish as compared with mammals (Saurabh & Sahoo, 2008). Immune-related enzymes, such as lysozyme and peroxidase, are part of the main innate immune parameters present in mucus, lymphoid tissue, plasma and other body fluids of freshwater fish. Our field study reveals that peroxidase activity decreased with increasing levels of metabolites (Figure 5.4A), but we did not observe such a relationship between organic contaminants and lysozyme activity. It is well established that the immune system of fish can be severely affected by various stress conditions. However, the nature of modulation by environmental contaminants is complex. Indeed, environmental contamination seems to have either stimulating or suppressing effects on innate immunity of fish. For instance, plasma lysozyme activity decreased in flounder contaminated with DDT adducts and PCBs (Skouras et al., 2003) whereas no modification was detected in sea bass exposed to heavy fuel oil (Bado-Nilles et al., 2011). These differences of responses

could be explained by the type and the concentrations of contaminants. Alternatively, contaminants could induce a short-time impact on the immune system. Dupuy et al., (2014) experimentally demonstrated a restoration of the lysozyme activity towards the initial levels in the European flounder, *Platichthys flesus*, exposed to a mixture of PAHs and PCBs, after a recovery period (14 days). Another explanation for the decrease in peroxidase activity with increasing levels of metabolites is that individuals exposed to environmental pollution invest more energy to help support the cost of detoxification (Du et al., 2019) at the expense of immunity (Dunier, 1996). This is indeed consistent with the liver enlargement previously observed in response to increasing PAH levels (Figure 5.1D). Likewise, EROD activity in chub liver significantly increased as a result of PCB exposure (Figure 5.9B), which has been recognized as a sensitive indicator of the inductive response of the cytochrome P450 system (Hewitt et al., 1998; Whyte et al., 2000).

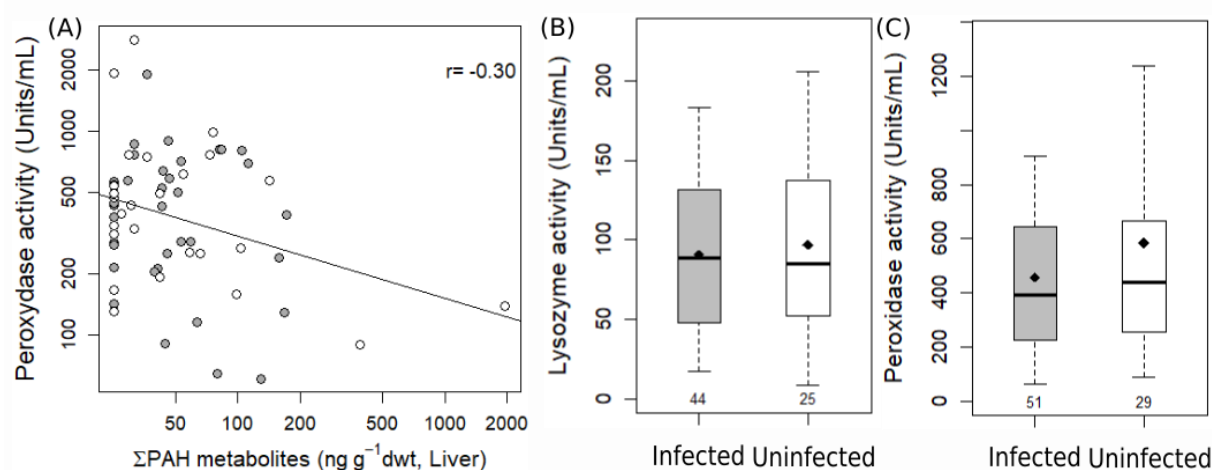


Figure 5.4. Effect chemical exposure and of parasite infection (grey: infected, white: uninfected) on the (A, C) peroxidase and (B) lysozyme activity (Units/mL) of chub under natural settings in 2016. Data are expressed as the mean \pm standard deviation. Numbers represent sample size.

The immunosuppressive effects of pollutants are also important in the dynamic of host-parasite interactions. One may predict that chemical-related immunosuppressive effects would enhance the probability of the parasite to successfully infect its definitive host. Indeed, it is well accepted that increases in chemical stress can result in increased susceptibility to microbial pathogens and other microparasites due to the immunosuppressive effect of pollutants (Sanchez-Ramirez et al., 2007). However, innate immune defenses, as measured by peroxidase and lysozyme

activity, did not differ between infected and uninfected hosts in the present study (Figures 5.4B and 5.4C). Cornet et al., (2009) previously investigated the effect of acanthocephalan parasites (*Pomphorhynchus laevis*, *Pomphorhynchus tereticollis* and *Polymorphus minutus*), on the immune response of their intermediate crustacean host. The authors demonstrated a causal relationship between parasite infection and differences in immune levels. Indeed, because of parasite infection, infected hosts had a lower level of immune defense. In our study, although some confounding factors may have blurred the effect of parasite infection on both lysozyme and peroxidase activity, suppression of the innate immune system would have led to an enhanced and chronic inflammatory response due to the parasites (Sorci & Faivre, 2009), ultimately increasing levels of oxidative stress. As we shall see below (2.2 *Oxidative status*), this was not the case. Consequently, the effect of *Pomphorhynchus* sp. on the immune system is likely to be host-specific. A possible explanation for the lack of effect on humoral components may be related to the immune activation by *Pomphorhynchus* sp., which may have been too low to trigger clear differential responses between infected and uninfected chubs. In this study, mono-infection with acanthocephalans alone in the intestinal tract and body cavity of chub was found while it was suggested that co-infections could indeed impose further selective pressure and a higher stress on the host than monospecific infections (Roon et al., 2015). In the end, therefore, our study did not confirm the synergetic effect of contaminant exposure and parasite infection previously reported in the literature (Morley et al., 2006; Sanchez-Ramirez et al., 2007; Marcogliese & Pietrock, 2011) and further investigations conducted with an experimental approach are thus needed.

2.2 *Oxidative status*

The oxidative status (defined as the amount of pro-oxidants and antioxidants that occur in cells/tissues) plays a central role in biological processes and its regulation is thought to be a major physiological mechanism underlying the capacity of organisms to cope with new environmental conditions (Beaulieu & Costantini, 2014). When oxidative species production overcome antioxidant systems (enzymatic and non-enzymatic components), oxidative stress arises, which can result in critical levels of oxidative damage to biomolecules (Soltani et al., 2019). Markers of oxidative status are thus important physiological parameters to assess the deleterious effect that organisms undergo when facing stressful conditions. One of the key findings in this thesis was that the oxidative status of chub was related to parasitic infection in chemically altered environments. Under natural conditions, ROM concentrations were

significantly lower in infected chubs compared to uninfected fish (Figure 5.5A), but were not related to the levels of contaminants.

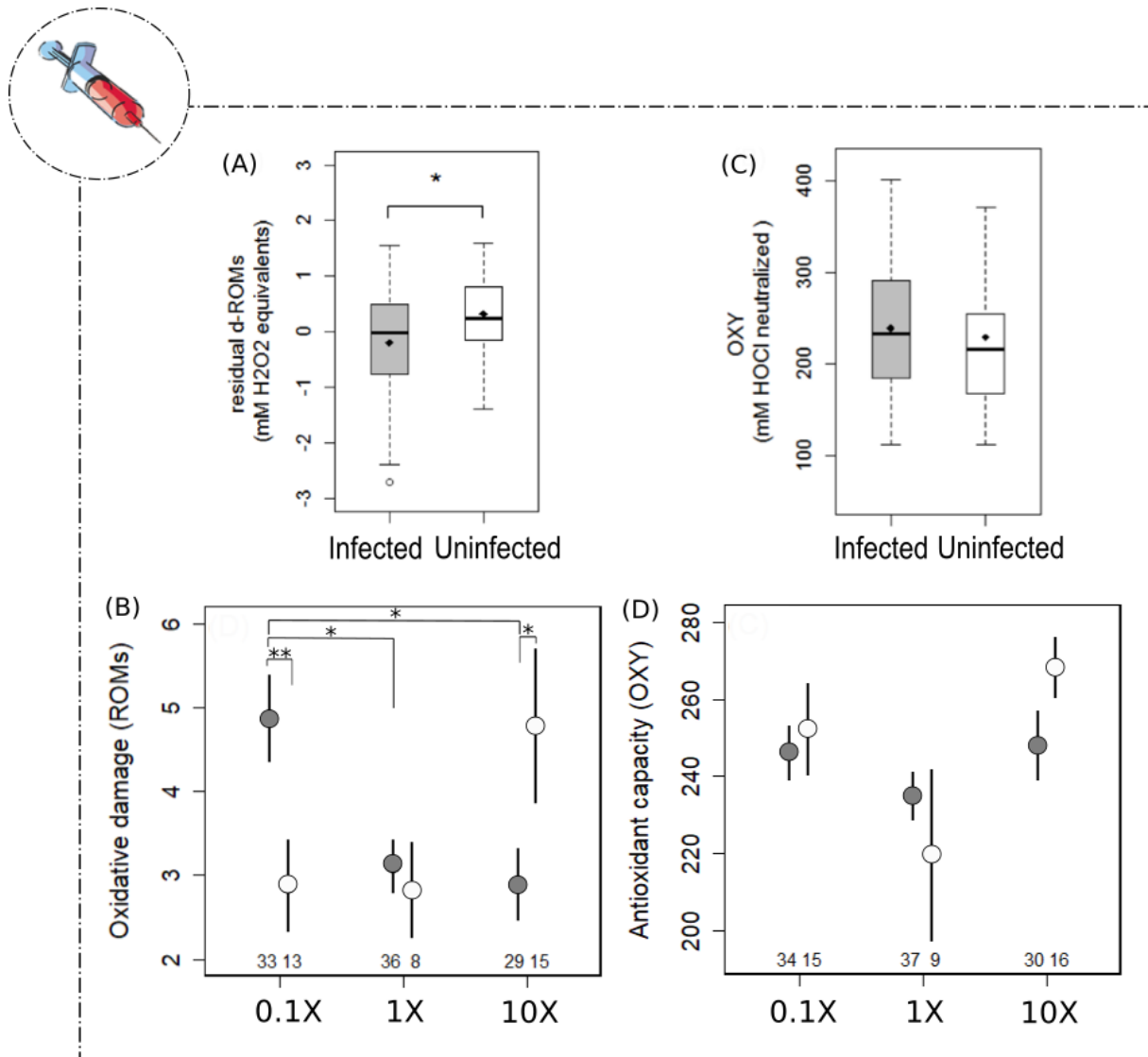


Figure 5.5. Effect of parasite infection (grey: infected, white: uninfected) and pollutant exposure on the oxidative damage (ROMs) and total antioxidant capacity (OXY) of chub under natural settings in 2016 (**A**, **B**) and after five-week experimental exposure to PAHs in 2019 (**C**, **D**). Data are expressed as the mean \pm standard deviation for (**A**, **B**) and as the mean \pm standard error for (**C**, **D**). Numbers represent sample size. Asterisks indicate significant effect of treatment (*: $p < 0.05$; **: $p < 0.001$).

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 & Costantini, 2014). Hydroperoxides are thus perceived as the earliest markers of oxidative damage in a biological system. This positive effect of parasites on host physiology (oxidative status), has been attributed to the capacity of intestinal parasites to accumulate toxicants and

deplete their host (Morrill et al., 2019, Article 2), therefore reducing reactive oxygen species (ROS) production or activated metabolites associated with chemical exposure. Previous findings also evidenced that parasites were beneficial to crustacean intermediate hosts exposed to pollution, by increasing antitoxic defenses (Gismondi et al., 2012; Sánchez et al., 2016), 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 contaminants in fish (Sures & Siddall, 1999; Brázová et al., 2012). Consequently, intestinal parasites may mitigate the production of highly reactive molecules generated from parent pollutants, thereby lowering toxicity (Morrill et al., 2019). This explanation is however not fully supported by our results, since infected and uninfected chubs did not differ in their pollutant (including metabolites) load (see Chapter IV). Contradictory studies stated that fish exposed to contaminants experienced greater stress when infected by parasites (Jacobson et al., 2003; Defo et al., 2019; Lacaze et al., 2019). For example, Marcogliese et al., (2005) showed enhanced sublethal stress effects (as measured by lipid peroxidation) in fish exposed to both inorganic contaminants and parasites. This effect was however only significant at higher infection levels.

As an inevitable consequence of analysing field data, potential confounding factors may have introduced a variety of unknown additional ecological or environmental factors contributing to the effects seen. For instance, 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 (Li et al., 2014). For that purpose, we conducted an experimental approach in 2019 on a wild chub population by controlling the exposure levels and the quality of their surrounding environments. After five weeks exposure to PAHs, variation in plasmatic ROM concentrations was explained by the interaction between infection status and treatment group (0.1X, 1X and 10X, see Chapter II – Data collection – *Experimental approach*). Parasitized chubs exposed to 0.1X displayed 41% higher ROM concentrations than uninfected ones (Figure 5.5B). In addition, ROM concentrations were significantly higher in parasitized chubs exposed to 0.1X compared to 10X and to a lesser extent 1X. These results likely reflect the cost of infection by *Pomphorhynchus* sp. on its final host. In response to infection, generation of ROS is one of the associated host immune reactions put forward (Shekhova, 2020). ROS either damage pathogen’s DNA through distortion of bases or activate pro-inflammatory cytokines (*i.e.*, involved in the up-regulation of inflammatory reactions), thus creating an unsuitable environment for the pathogen. This assumption is further supported by the significantly higher abundance of the *Firmicutes* phylum in the intestine of

infected chubs (3.38 ± 7.49) compared to uninfected ones (0.03 ± 0.04). *Firmicutes*, specifically taxa including *Tyzzarella*, are systematically associated to intestinal inflammation in Humans (Qiu et al., 2017; Olaisen et al., 2020). At 1X concentration, no difference in ROM levels was observed between chubs infected or not by intestinal worms. While the physiological cost of parasite infection likely persists, worms could start to accumulate PAHs thereby reducing pro-oxidant compounds produced through the biotransformation processes. At 10X, ROM concentrations in chub were 39% lower in infected individuals compared to uninfected ones. With increasing contaminant exposure, the positive effects of intestinal worms, through their bioaccumulation capacities, could outweigh their negative effects. Thus, the outcome of host-parasite interactions appear to be context dependent, indicating that the effect of *Pomphorhynchus* sp. on its fish host can range from negative to positive as organic pollutant exposure increases. A noteworthy point is that parasites also possess an antioxidant system, which can be understood as a means to avoid and bypass immune responses of the host. Specifically, parasites possess glutathione peroxidase (Radovanović et al., 2015), which detoxifies organic hydroperoxides formed during oxidative stress. It would have been interesting to quantify antioxidants in parasite tissues to determine if their activity increases with increasing PAH exposure, potentially helping antitoxic defenses of the host (Sures & Radszuweit, 2007; Morassutti et al., 2011). Nevertheless, total plasma antioxidant capacity (OXY) and superoxide dismutase, a first-line defense against oxidative stress, were unrelated to the presence of intestinal worms in our field study (LMM: $F_{(1,68.9)}=0.320$, $p=0.573$; Figures 5.5A and 5.6A, respectively) but were negatively associated with increasing levels of parent organic pollutants (LMM: $F_{(1,73.9)}=10.02$, $p=0.002$; Figure 5.6C) and their metabolites (Figure 5.6D), indicating a challenged antioxidant capacity. This decrease in antioxidant defenses in fish exposed to environmental pollution has been well document in the literature (Wilhelm Filho et al., 2001; Qu et al., 2015). It is hypothesized that ROS can deplete endogenous antioxidants by inducing oxidative stress, through xenobiotic biotransformation, and/or down-regulate their transcriptional expression (Cong et al., 2020).

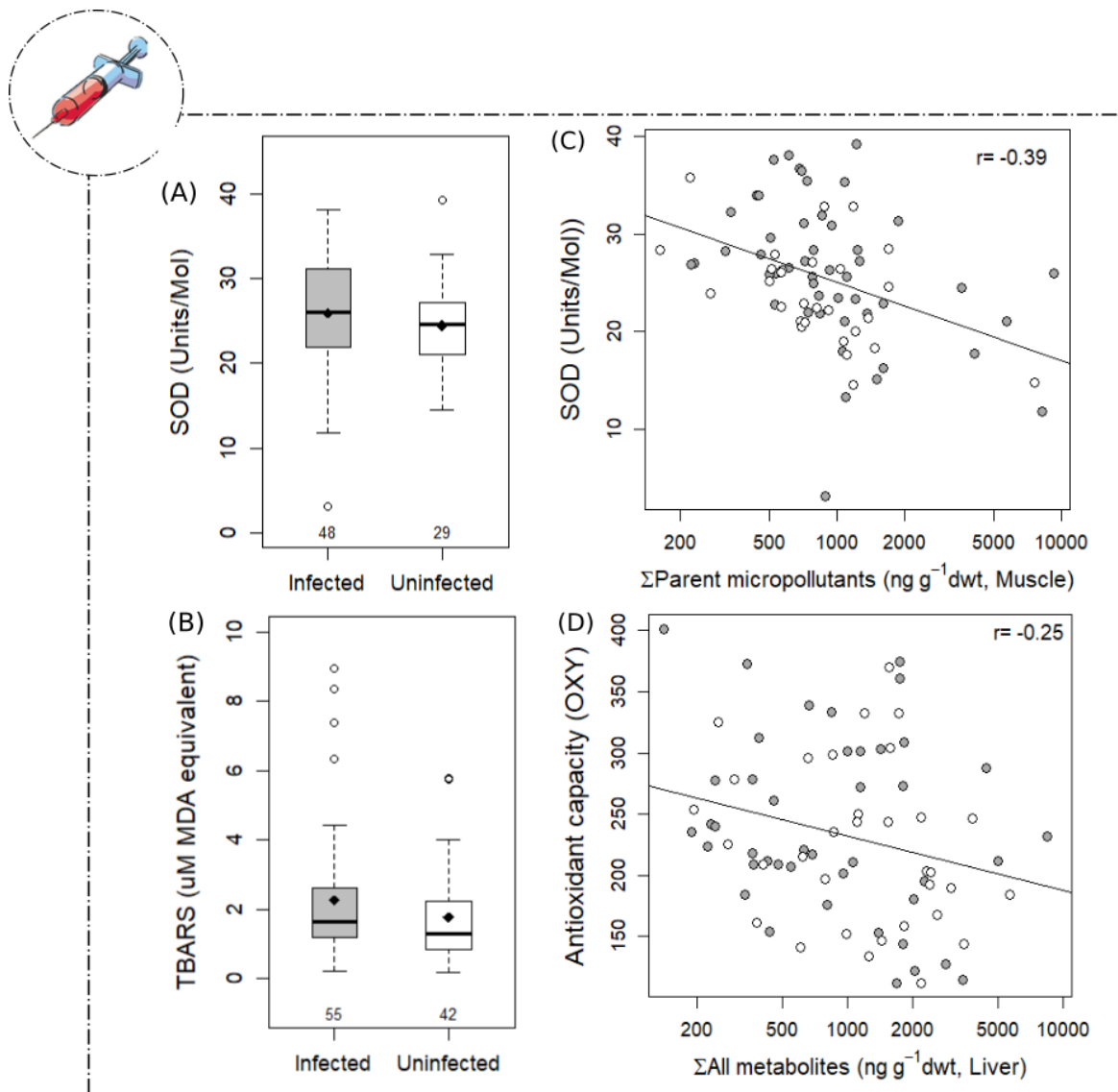


Figure 5.6. Effects of both parasite infection on (A) Superoxide Dismutase (Units/Mol) and (B) Thiobarbituric acid reactive substances, and organic contaminant exposure (Σ parent compounds or their metabolites) on (C) SOD and (D) total antioxidant capacity of chub under natural settings in 2016. TBARS assays provides a general quantification of oxidative damage molecules that is very sensitive to exposure of the organism to environmental stressors.

Interestingly, even though uninfected chubs had higher ROM concentrations (early stage of lipid peroxidation), antioxidant defenses seem to be sufficient to avoid the establishment of an oxidative stress condition, as indicated by the lack of changes in TBARS (late stage of lipid peroxidation; LMM: $F_{(1,88,4)}=1.328$, $p=0.252$; Figure 5.6B) between infected and uninfected hosts. Similarly, in 2019, total plasma antioxidant activity, did not significantly differ among treatment groups, nor between infected and parasitized-free individuals (Figure 5.5D). 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 contaminant exposure on antioxidant defences given that fish were not constrained with regard to antioxidant availability. Recently, [Sánchez et al. \(2016\)](#) highlighted higher levels of antioxidant defences (catalase and glutathione reductase) in infected invertebrates, reflecting a better ability to counteract the oxidative effects of organic pollutants.

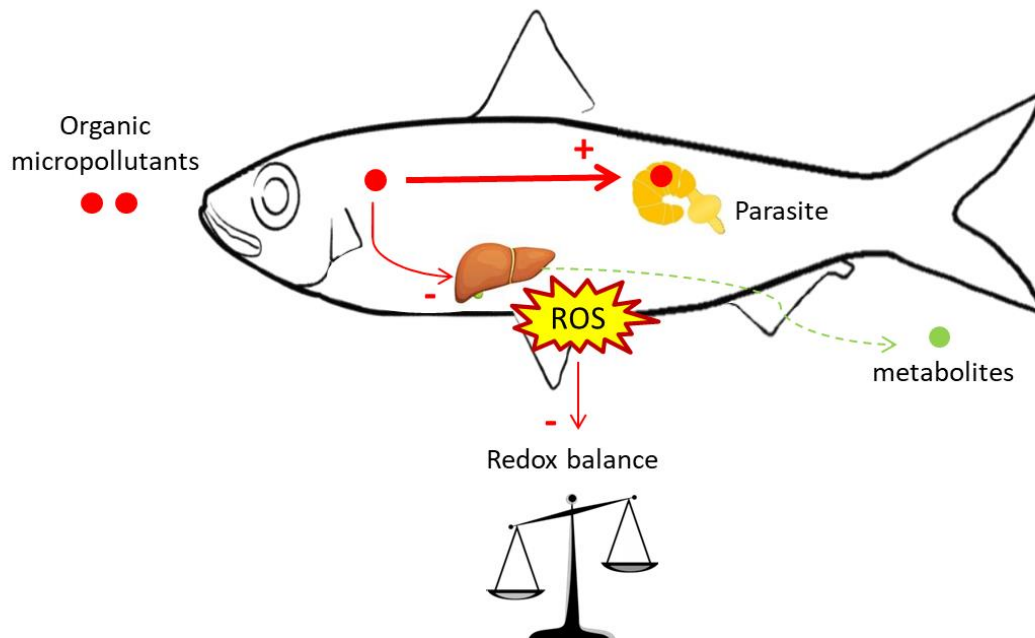


Figure 5.7. Potential underlying mechanisms explaining the reported shift in oxidative damage, between infected and uninfected wild chub (*Squalius cephalus*), with increasing pollutant load. ROS: reactive oxygen species.

Overall, our results support the hypothesis that acanthocephalans are conditionally helpful parasites for chub under polluted conditions ([Weinersmith & Earley, 2016](#)) as enhanced oxidative damage, and potentially oxidative stress, in unparasitized chub could drive fitness costs for the host ([Figure 5.7](#)). Consequently, intestinal worms such as *Pomphorhynchus* sp. could enhance the capacity of host populations to persist in polluted environment, so that a high prevalence of these parasites could be perceived as an adaptive response to contaminants for successful maintenance of homeostasis. Indeed, while the prevalence of *Pomphorhynchus* sp. in the different fish populations was high (2016: 50-90 % and 2019: 60-80 %), the abundance and infection intensity of these parasites was fairly low (2019: 1-11 parasites; not measured in 2016).

2.3 Telomeres

Through their adverse effects, environmental stressors (parasite infection and chemical exposure) may ultimately lead to fitness consequences, such as reduced animal's survival.

Combined effects of environmental contaminants and parasite infection on telomere length are however almost unknown for wildlife, especially fish species (Stauffer et al., 2017). In this thesis, we observed a significant difference in age-corrected relative telomere length (RTLc) of chubs between urban and agricultural habitats (Figure 5.8A), with telomeres ~ 10 % longer in fish near agricultural areas than those closest to Paris, at proximity to urban habitats.

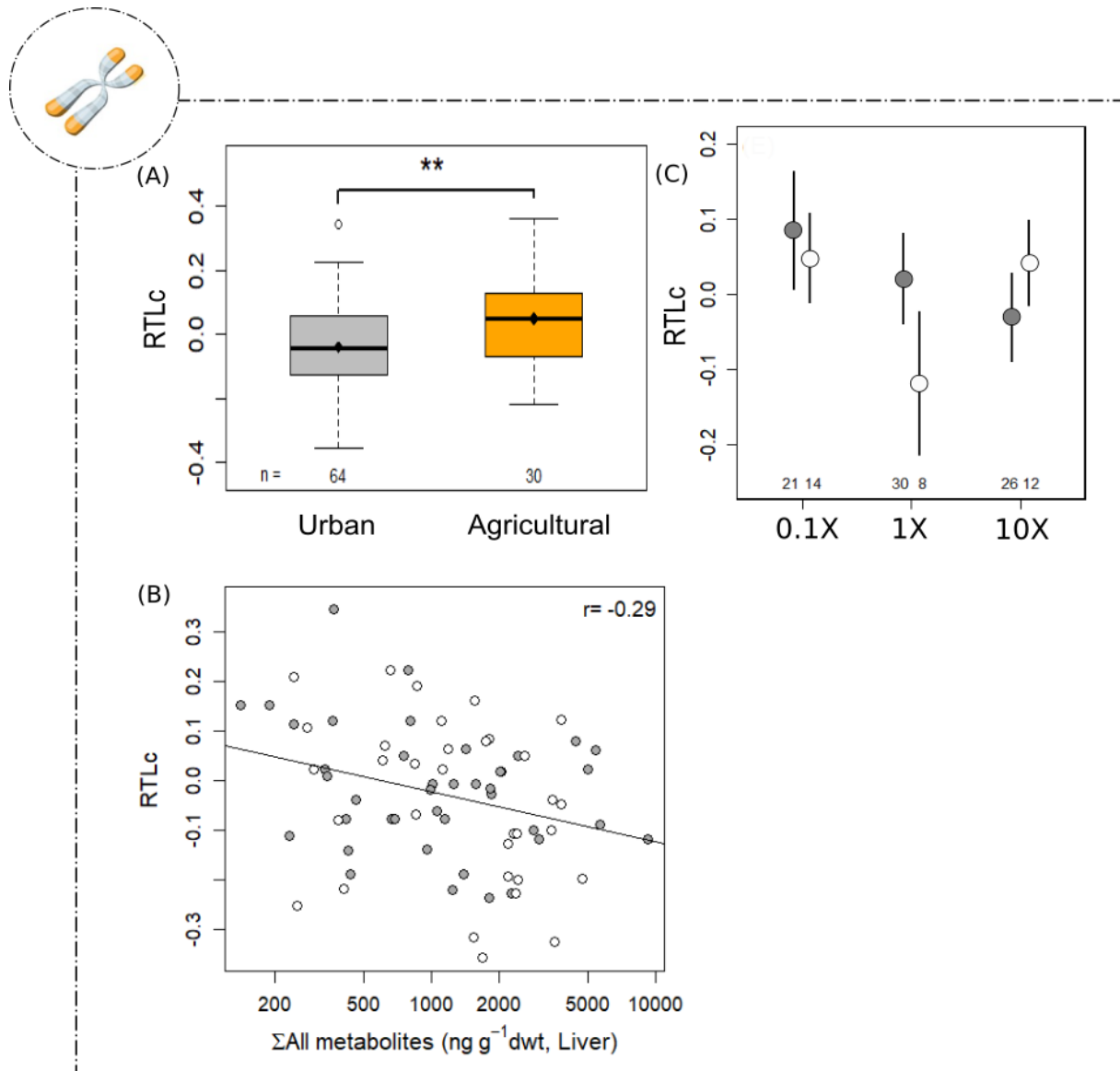


Figure 5.8. Effect of parasite infection (grey: infected, white: uninfected) and pollutant exposure on age- or length-corrected telomere length (RTLc, residuals $RTL \sim \text{age/length}$) of chub under natural settings in 2016 (A, B) and after five-week experimental exposure to PAHs in 2019 (C). Filled diamonds represent the arithmetic mean. Numbers represent sample size. Data are expressed as the mean \pm standard deviation for (A) and as the mean \pm standard error for (C). ** indicates a significant difference ($p < 0.001$)

As previously found in birds (Meillere et al., 2015; Salmón et al., 2016; Ibáñez-Álamo et al., 2018; Grunst et al., 2020), telomeres were shorter in urban habitats compared to agricultural ones, suggesting higher life-threatening situations for fish in urban rivers. Additionally, RTLc was found to significantly decrease with increasing levels of pollutants, especially for metabolites of organic contaminants (Σ all metabolites, Figure 5.8B) and PCBs. To date and to the best of our knowledge, this work is the first evidence that organic pollutants, especially metabolites, negatively impact telomere length in fish. Therefore, no comparisons with previous studies are possible and our findings can only suggest a potential negative effect of chemical by-products on telomeres in the European chub. In different species of birds, exposure to environmental contaminants (OCPs, perfluoroalkyl substances: PFAS and trace metals) was associated with a general reduction in telomere length (Blévin et al., 2016; Stauffer et al., 2017; Louzon et al., 2019). However, no significant relationships were observed between absolute telomere length and organohalogenated contaminants (including OCPs and PCBs) in white-tailed eagle chicks (*Haliaeetus albicilla*) from northern Norway (Sletten et al., 2016). First, low contaminant loadings may explain the lack of significant relationship on telomere length, (e.g., $\Sigma 9$ PCBs: 35.0 ± 4.96 ng g⁻¹ wet weight, median: 24.3 ng g⁻¹ ww) compared to our study ($\Sigma 7$ PCBs 107 ± 148 ng g⁻¹ dry weight, median: 64.7 ng g⁻¹ dwt). Second, birds and fish species differ on their telomere length maintenance pathways that are key to protecting and stabilizing the genome against various stressors, including pollutants. It is worth noting that fish from urban and agricultural rivers did not differ in their pollutant load, except for slightly higher levels of metabolizable pollutants in urban watercourses, such as plasticizer (urban: 799 ± 1082 ng g⁻¹ dwt; agricultural: 557 ± 1389 ng g⁻¹ dwt) and pyrethroids (urban: 509 ± 1411 ng g⁻¹ dwt; agricultural: 387 ± 541 ng g⁻¹ dwt). Urban river systems have however undergone profound changes, such as damming, banking and channelization that have led to the disruption of longitudinal connectivity, loss of wetlands and spawning grounds, but also increased water temperature, pathogens and boat noise (Paul et al., 2001; Hanache et al., 2020). Our correlative approach suggest that the diverse and profound degradation of urban streams induce deleterious effects in fish by accelerating telomere attrition and probably jeopardizing their survival. Those results are in line with previous findings, stating that environmental stressors accelerate telomere shortening in avian and fish species (Jasinska et al., 2014; Stauffer et al., 2017; Angelier et al., 2018).

Some underlying mechanisms may explain the negative correlation between pollutants and telomere length. Under natural conditions, metabolites of organic pollutants were

negatively correlated with total antioxidant capacity (OXY) and peroxidase activity but not to oxidative damage in chub plasma (Figures 5.6D and 5.4A).

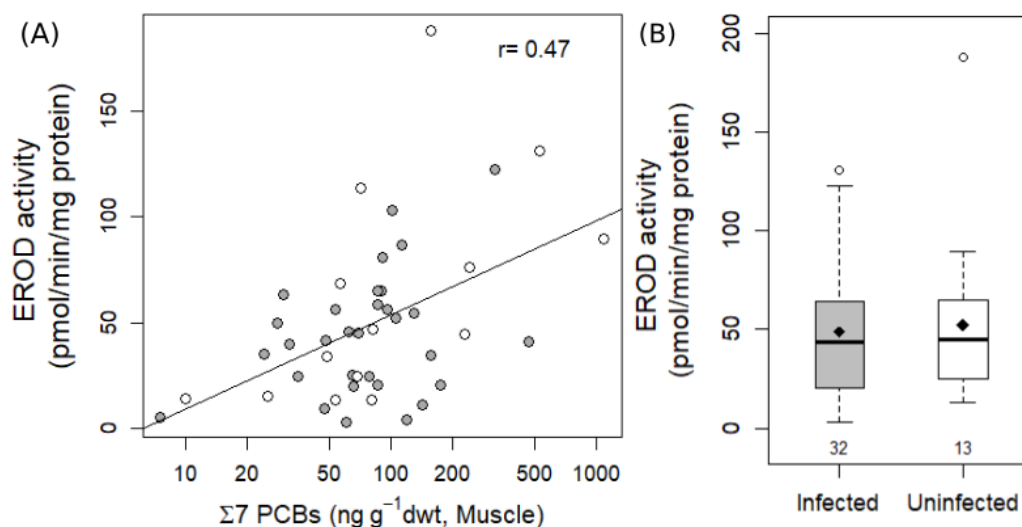


Figure 5.9 Effect of (A) chemical exposure and (B) parasite infection on EROD activity (pmol/min/mg protein) of chub under natural settings in 2016.

Similarly, increasing levels of PCBs were positively correlated with EROD activity (LMM: $F_{(1,30.1)}=14.87$, $p<0.001$; Figure 5.9A), oxidative damage (TBARS; LMM: $F_{(1,72.2)}=4.695$, $p=0.033$), and associated to shorter telomeres. In addition, intestinal parasites had no effect on the activity of xenobiotic-metabolizing enzymes (EROD; LMM: $F_{(1,37.9)}=0.357$, $p=0.554$; Figure 5.9B). Even though the underlying mechanisms are still poorly understood, we hypothesize that electrophilic intermediates generated through the biotransformation of parent compounds could increase oxidative attacks and deplete or weaken defence mechanisms (*i.e.*, antioxidants), ultimately shortening telomeres (Figures 5.10 and 5.11A).

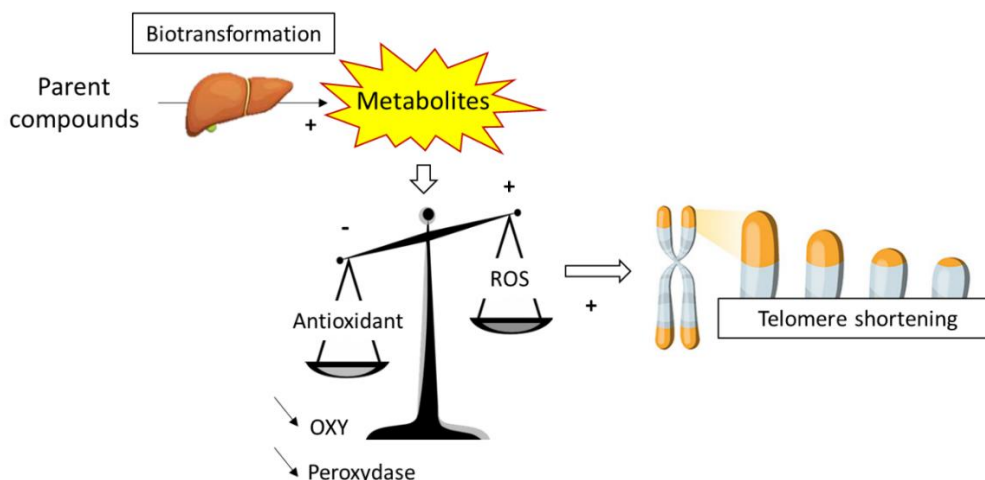


Figure 5.10. Potential underlying mechanisms explaining the reported relationship between the levels of metabolites of organic pollutants (Σ , ng g⁻¹ of dry weight, Liver) and age-corrected

telomere length (RTLc, residuals RTL ~ age) of wild chub (*Squalius cephalus*). ROS means reactive oxygen species and OXY refers to assays measuring total antioxidant capacity in plasma samples.

Among DNA bases, the guanine is more prone to oxidation due to having a low redox potential. The telomere repetitive sequences (TTAGGG)*n* shared by vertebrates are therefore particularly vulnerable to oxidative attacks (Singh et al., 2019). Alternatively, DNA repair mechanisms also greatly influence telomere length and dynamics. Involved in *de novo* telomere synthesis, the enzyme telomerase partially compensates telomere shortening throughout life by adding new telomeric sequences onto the ends of chromosomes at each DNA replication. This enzyme, a reverse transcriptase, has been shown to be the primary mechanism for telomere maintenance and genomic integrity (Jaskelioff et al., 2011). In contrast to birds and mammals, fish retain telomerase expression across tissues during adulthood (Hatakeyama et al., 2016). Given that PCBs exposure has been shown to reduce telomerase activity (Xin et al., 2015), environmental pollutants could induce a down-regulation of telomerase activity (Figure 5.11B). In this thesis, telomerase enzyme activity was not measured. Thus, further investigations measuring at the same time organic contaminants (parent compounds and their metabolites), telomerase activity and telomere length are needed to test if reduced telomerase activity induced by contaminant exposure could be linked to telomere shortening.

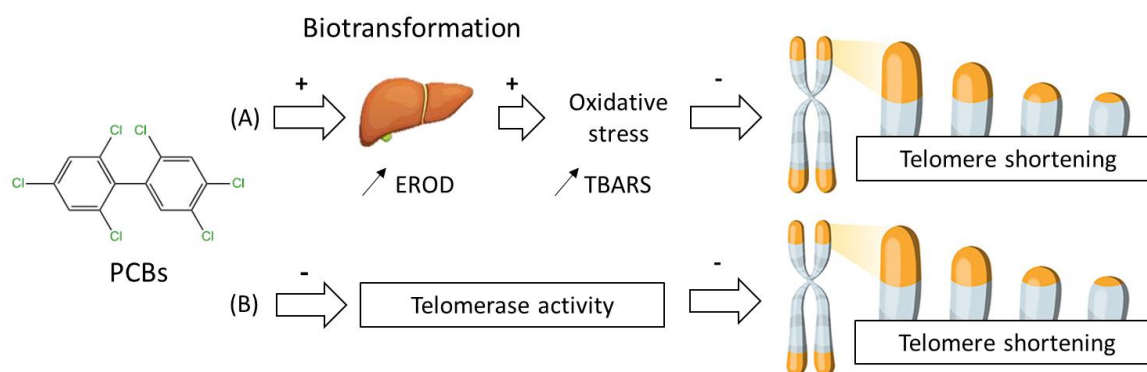


Figure 5.11. Potential underlying mechanisms explaining the reported relationship between the levels of PCBs (Σ 7 PCBs, ng g⁻¹ of dry weight, Muscle) and age-corrected telomere length (RTLc, residuals RTL ~ age) of wild chub (*Squalius cephalus*). EROD: Etoxyresorufine-O-deethylase, TBARS: Thiobarbituric acid reactive substances and PCBs: Polychlorinated biphenyl.

Importantly, we did not observe any significant effect of parasite infection on telomere length, neither under natural settings nor controlled conditions, although uninfected hosts seemed to

have shorter telomere length, potentially reflecting lower individual quality (Figure 5.8C). While infected individuals usually experienced a significantly greater rate of telomere shortening than uninfected individuals (Asghar et al., 2015, 2016; Karell et al., 2017), this lack of effect has already been reported in fish species (Stauffer et al., 2017) and adds to the evidence that parasitic infections do not always entail negative consequences for the host. However, we could not exclude that some confounding factors may have blurred the effect of parasite infection on the relative telomere length of chubs. For instance, the effect of host interaction with its intestinal parasite may be sex-specific (Sudyka et al., 2019). Indeed, fish sex is known to affect parasitism and potentially also accumulation of pollutants (Madenjian et al., 2016). Alternatively, the effect of parasitic infections on telomere length may stem from different phases of infection. Previous studies have reported a consistent pattern of a faster rate of telomere shortening subsequent to infection. In birds, malaria-infected siskins, *Spinus spinus*, had substantially shorter telomeres compared with controls at 105 days post-infection (Asghar et al., 2016). Similarly, in humans, malaria infection considerably shortened telomeres up to 3 months post infection, but telomere length was restored after 1 year (Asghar et al., 2017). In our field study and experimental approach, telomeres were only measured once, so that our temporal resolution may obscure potential parasite effects. However, studying the effect of different phases of infection on the host remains complex to carry out with wild populations since hosts are often simultaneously exposed to multiple life cycle stages of the parasite (e.g., cystacanths and adult worms, see **Chapter II – Chub-acanthocephalan model**).

It worth noting that the physiological costs (telomere length) to fish exposed to increasing organic contaminants under natural settings, was not confirmed by the experimental approach on wild chubs (Figure 5.8C). Indeed, while experimental exposure to PAHs affected the oxidative status of chub, no statistically significant effects were detected on the telomere length. 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 contaminant exposure and parasite infection on telomere length when using a cross-sectional approach. In line with these results, a previous study reported no relationships between perfluoroalkyl substances (PFASs) and absolute telomere length in black-legged kittiwakes, *Rissa tridactyla*, when analyzing only one year, but PFASs were good predictors of differences in telomere length between years (Blévin et al., 2017). However, both elongation and shortening in telomere length was observed among

individuals from the same population exposed to same class of contaminants (Blévin et al., 2017; Sebastiano et al., 2020), which complicates interpretation of the results. For that purpose, studies should be focused on the effect of both chemical exposure and parasite infection on telomere dynamics in wild animals. In this PhD work, this was not investigated considering the limited time window of our experimental approach (5 weeks) compared to the longevity of the species in the wild (up to 20 years). Additionally, while stressful environmental conditions can affect telomere length in adults, most telomeres shortening occurs early in life, during growth (Simide et al., 2016).

Although telomeres are highly sensitive to oxidative damage, which can accelerate telomere shortening (Geiger et al., 2012; Reichert & Stier, 2017; but see Boonekamp et al., 2017) we detected no associations between those two physiological markers, whatever the approach (Figure 5.12, In 2016, LMM: $F_{(1,66)}=0.055$, $p=0.815$). Other authors were unable to clearly establish a link between high levels of oxidative damage and exacerbate telomere shortening in zebra finches, *Taeniopygia guttata* (Reichert et al., 2014), in Adélie penguin, *Pygoscelis adeliae* (Beaulieu et al., 2011) or in brown trout, *Salmo trutta* (Stauffer et al., 2017). These findings suggest that for the moment the link between oxidative status and telomere erosion is complex to assess *in vivo* and call for further evidence.

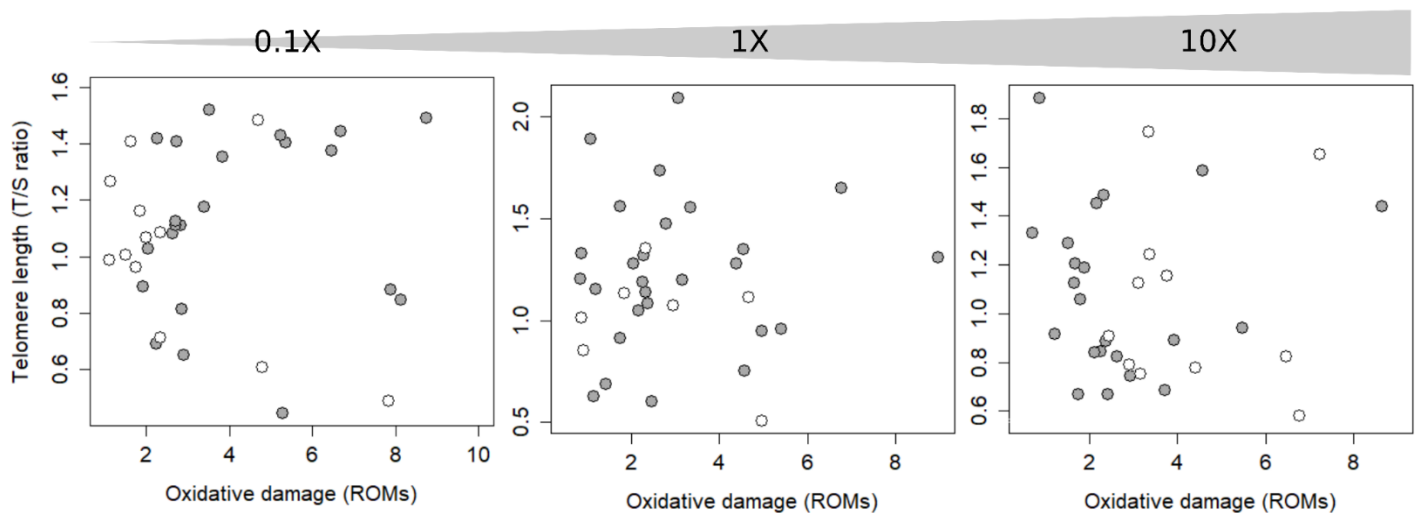


Figure 5.12. Association between oxidative damage (ROMs, mM H₂O₂ equivalents) and telomere length (T/S ratio) of chub exposed to different concentrations of PAHs (0.1X, 1X and 10X) depending on the presence of parasite *Pomphorhynchus* sp. (grey: infected and white: uninfected).

VI – Conclusion, limits and perspectives

The main focus of the current thesis was to extend the knowledge about the eco-physiological consequences of organic contaminant exposure and parasite infection on aquatic vertebrates, with a focus on several legacy POPs, emerging or semi-persistent contaminants and some of their metabolites, still poorly investigated in wildlife ecotoxicology. On one hand the research emphasis was aimed at examining biomarker responses and contaminant body burden in relation to several individual traits of chub, *Squalius cephalus* (**Chapter III**). On the other hand the thesis evaluated the capacity of the acanthocephalan *Pomphorhynchus* sp. to bioaccumulate different classes of organic contaminants as well as their impact on the contaminant body burden of the host (**Chapter IV**). Finally, the thesis examined the eco-physiological responses to both stressors at different levels of biological organization (**Chapter V**), which was performed using complementary approaches (correlative and experimental) on wild populations of European chub. The main results are illustrated in **Figure 6.1**.

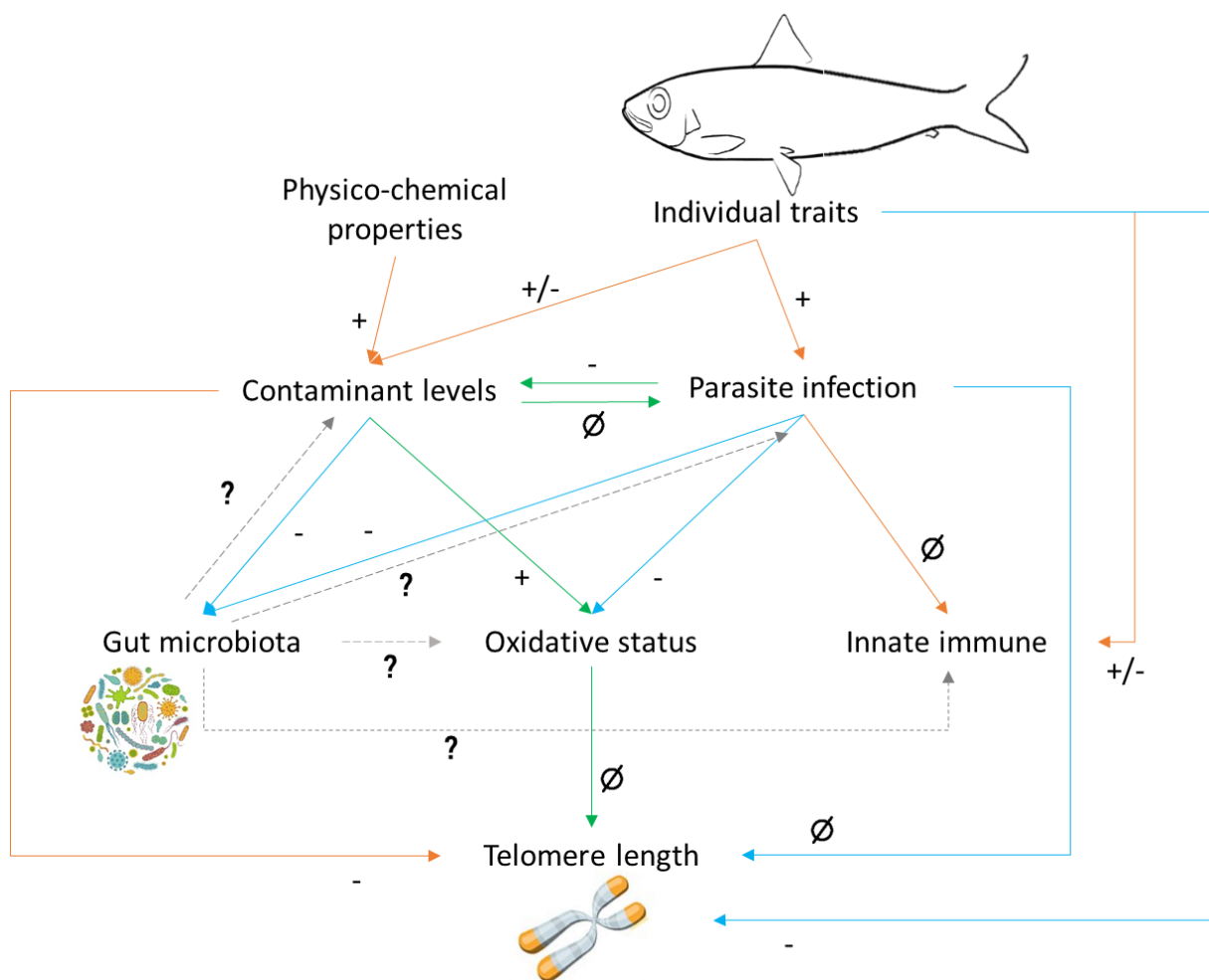


Figure 6.1. Schematic representation of the relationships found in the thesis between individual traits, contaminant exposure and properties, parasite infection and biomarker responses, from

the correlative study (orange arrow), the experimental approach (blue arrow) or from both approaches (green arrow). Full line indicates effects studied in the thesis and dot line possible link that should be investigated. Results on the hepatosomatic index and Fulton's condition are not represented.

We first demonstrated in this thesis that biological and ecological factors are key parameters to understand the fate of environmental contaminants as well as the biochemical and physiological responses of organisms to chemical stress. Although these relationships are hard to predict, especially under natural conditions, we showed that results generated under a field study and an experimental approach were highly consistent. Importantly, chemical properties, in particular the metabolic biotransformation rate, were found to explain the distribution of contaminants inside host-parasite systems. The accumulation of organic contaminants in intestinal parasites demonstrates that these chemicals are biologically available and that parasites should be viewed as potential confounding factors in exposure assessments to environmental contaminants since their presence might modify chemical concentrations in fish tissues. When applicable, parasites should therefore be considered complementary to chemical analysis to evaluate the exposure of organisms to organic contaminants in freshwater ecosystems. Although similar findings have been mostly reported in fishes, the capacity of intestinal parasites (mainly cestodes and acanthocephalans) to accumulate toxicants has been observed in sharks (Malek et al., 2007), rats (Torres et al., 2011) and red foxes (Jankovská et al., 2010; Borkovcova et al., 2020), so that they might have vast applications in the aquatic (freshwater and marine) and terrestrial ecosystems. It worth noting that with the use of naturally infected fish populations, we could not exclude that the pollutant load in *Pomphorhynchus* sp. might result from previous exposure in the intermediate host (*i.e.*, in gammarids). However, pooled samples of *Pomphorhynchus* sp. cystacanth showed similar patterns of contamination by organic pollutants than their intermediate gammarid host and contaminant levels in cystacanths were up to 10 times lower than those reported in the definitive host collected under natural conditions (in 2016). Additionally, Sures & Siddall (1999) reported that chemicals predominantly accumulated in acanthocephalans inside the definitive host. Importantly, while body burdens did not significantly differ between infected and uninfected fish, nor affected biotransformation processes, levels of PAHs in infected chubs decreased with increasing parasite load, which confirms their detoxifying ability.

The last chapter especially underlines the toxicity of contaminant by-products, which highlights the importance of considering their effects to better assess the impacts of currently-released chemicals on wildlife. Specifically, contaminant by-products were associated with

increased oxidative damage, reduced antioxidant defenses and shorter telomeres, a proxy of life expectancy, in wild populations of a common freshwater fish, the European chub. Although uninfected fish displayed higher oxidative damage, and even though a typical pathway of chemical toxicity is mediated through the increased intracellular generation of pro-oxidant compounds, we could not clearly determine the outcome of such an imbalance in the oxidative status. These results call for further investigations on both gut microbiota, to assess the fitness consequences of the altered gut bacterial communities, and on telomere dynamic. Overall, our results make some useful and original contributions regarding the impacts of human-induced pollution on aquatic wildlife and provide the first evidence that the outcome of host-parasite interactions in aquatic environments can shift from negative to positive as organic pollutant exposure increases (Figures 5.5B and 6.2). From a fitness' point of view, this potential positive effect of intestinal worms will be of advantage for the host in polluted environments if the latter is still able to grow and reproduce.

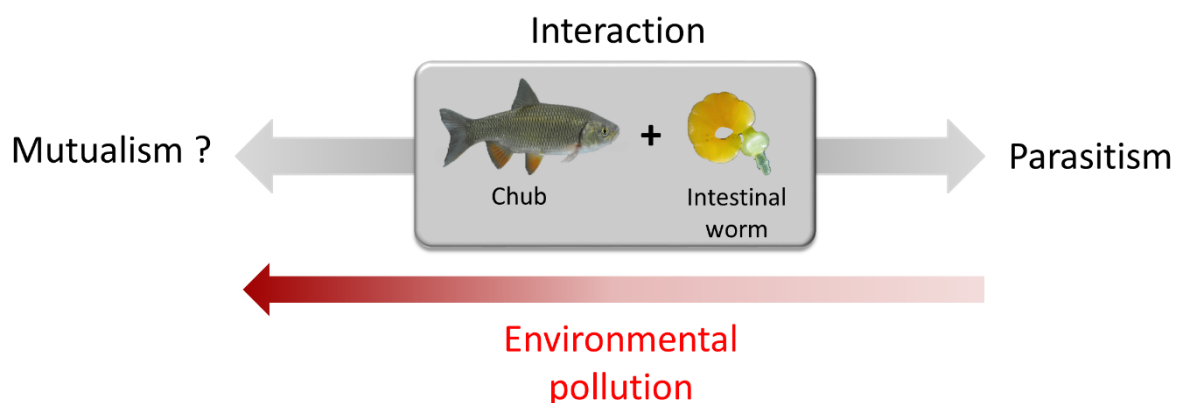


Figure 6.2. Potential shift from parasitism to mutualism due to environmental changes.

Individually, both contaminant exposure and parasitism can affect reproductive functions, such as the development of gonads in wild fish (Kime, 1995; Hecker & Karbe, 2005). It was demonstrated that the gonadosomatic index was significantly lower in infected female and male roach, *Rutilus rutilus*, and was associated with a pronounced disruption of the prime endocrine system regulating reproduction (Trubiroha et al., 2010). Consequently, we need to further evaluate this inter-specific relationship and, for instance, assess whether the combined effect of parasite infection and pollutant exposure affect life history traits of the host (growth and reproduction). At this point, our results suggest that intestinal worms had an effect on host health and that exposure to organic contaminants can modulate interspecific interactions. Additionally, the interaction between contaminants and parasite, as demonstrated in our

experimental approach on the oxidative status, highlight that parasites impact the response of key biomarkers. If not considered, parasites could affect the reliability of biomarkers as a diagnostic tool to assess the effects of environmental pollutants. A major limitation in our approaches, is that sex was not recorded. In light to physiological differences between both males and females (Vuorinen et al., 2006; Madenjian, et al., 2017), including sex in your analyses might perhaps reveal patterns that are now hidden in the data, not only for contaminant accumulation but also for biomarker responses. Thus, sex-specific effects between pollutant burden or biomarker responses and parasite infection have to be elucidate in the future. At last, while we evaluated the physiological costs of the combined effect of parasite infection and pollutant exposure on the fish host, it will be relevant to determine if the huge amount of chemicals accumulated in the intestinal worm affect its own reproduction. Indeed, although females *Pomphorhynchus laevis* highly contaminated by lead were able to release eggs containing acanthors (Sures et al., 1994), Sures et al. (2000) reported that acanthocephalans were able to discharge metals *via* the shells of their eggs. This detoxification pathway could potentially impair their hatchability (Gilbert & Avenant-Oldewage, 2016). Up to now, little is known about the sensitivity of parasite with complex life cycles to environmental pollution, especially organic chemical, but it could be a valuable information to help understand host-parasite dynamics in polluted environments. These complex interactions between parasites, their hosts and pollution are indeed still not fully understood and parasite-pollutant associations remain unclear. Depending on the taxonomic group and types of contaminants, parasites react differently to environmental changes, such as pollution (Lafferty, 1997). Contradictory findings have been reported regarding the effects of environmental pollution on parasite species abundance, composition and richness (Blanar et al., 2009; Vidal-Martinez et al., 2009). This comes from the fact that environmental pollution often involve complex mixtures and that the physiology of parasites probably varies among species or among life stages of individuals within species. Lastly, *Pomphorhynchus* sp. is vertically transmitted and has a lifespan of approximatively seven to eight months (Nachev & Sures, 2016). Thus, the question arises: “What happen to the host after parasite death?”. The hypothesis of a niche specialization may lead to a never ending infestation of the host, as current infections may increase host vulnerability to new infestation. In fact, wild chubs were often simultaneously exposed to multiple life cycle stages of the parasite (*e.g.*, cystacanths and adult worms). However, our work was not designed to answer this specific question, and future research will be necessary to test this hypothesis.

VII – References

- Ábalos, M.; Barceló, D.; Parera, J.; la Farré, M.; Llorca, M.; Eljarrat, E.; Giulivo, M.; Capri, E.; Paunović, M.; Milačić, R.; Abad, E. Levels of regulated POPs in fish samples from the Sava River Basin. Comparison to legislated quality standard values. *Sci. Total Environ.* **2019**, 647, 20–28. (doi:10.1016/j.scitotenv.2018.07.371)
- André, J. M.; Ribeyre, F.; Boudou, A. Mercury contamination levels and distribution in tissues and organs of delphinids (*Stenella attenuata*) from the Eastern Tropical Pacific, in relation to biological and ecological factors. *Mar. Environ. Res.* **1990**, 30, 43–72. (doi:10.1016/0141-1136(90)90010-L)
- Angelier, F.; Costantini, D.; Blévin, P.; Chastel, O. Do glucocorticoids mediate the link between environmental conditions and telomere dynamics in wild vertebrates? A review. *Gen Comp Endocrinol.* **2018**, 256, 99–111. (doi:10.1016/j.ygcen.2017.07.007)
- Angelier, F.; Weimerskirch, H.; Barbraud, C.; Chastel, O. Is telomere length a molecular marker of individual quality? Insights from a long-lived bird. *Funct. Ecol.* **2019**, 33, 1076–1087. (doi:10.1111/1365-2435.13307)
- Aguiar, L. S.; de Oliveira, M. I. B.; de Matos, L. V.; Gomes, A. L. S.; da Costa, J. I.; da Silva, G. S. Distribution of the acanthocephalan *Neoechinorhynchus buttnerae* and semiquantitative analysis of histopathological damage in the intestine of tambaqui (*Colossoma macropomum*). *Parasitol. Res.* **2018**, 117, 1689–1698. (doi:10.1007/s00436-018-5840-8)
- Aitzetmüller, K.; Taraschewski, H.; Filipponi, C.; Werner, G.; Weber, N. Lipids of fish parasites and their hosts: fatty acids of phospholipids of *Paratenuisentis ambiguus* and its host eel (*Anguilla anguilla*). *Comp. Biochem. Physiol. B* **1994**, 109, 383–389. (doi: 10.1016/0305-0491(94)90021-3)
- Arnot, J. A.; Gobas, F. A. P. C. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environ. Toxicol. Chem.* **2004**, 23, 2343–2355. (doi:10.1897/03-438)
- Asghar, M.; Hasselquist, D.; Hansson, B.; Zehtindjiev, P.; Westerdahl, H.; Bensch, S. Hidden costs of infection: Chronic malaria accelerates telomere degradation and senescence in wild birds. *Science* **2015**, 347, 436–438. (doi:10.1126/science.1261121)
- Asghar, M.; Palinauskas, V.; Zaghdoudi-Allan, N.; Valkiūnas, G.; Mukhin, A.; Platonova, E.; Färnert, A.; Bensch, S.; Hasselquist, D. Parallel telomere shortening in multiple body tissues owing to malaria infection. *P. Roy. Soc. B-Biol. Sci.* **2016**, 283, 20161184. (doi:10.1098/rspb.2016.1184)
- Asghar, M.; Yman, V.; Homann, M. V.; Sondén, K.; Hammar, U.; Hasselquist, D.; Färnert, A. Cellular aging dynamics after acute malaria infection: A 12-month longitudinal study. *Aging Cell* **2017**, 17, e12702. (doi:10.1111/accel.12702)
- Baali, A.; Kammann, U.; Hanel, R.; El Qoraychy, I.; Yahyaoui, A. Bile metabolites of polycyclic aromatic hydrocarbons (PAHs) in three species of fish from Morocco. *Environmental Sciences Europe* **2016**, 28. (doi:10.1186/s12302-016-0093-6)
- Bado-Nilles, A.; Quentel, C.; Mazurais, D.; Zambonino-Infante, J. L.; Auffret, M.; Thomas-Guyon, H.; Le Floch, S. In vivo effects of the soluble fraction of light cycle oil on immune functions in the European sea bass, *Dicentrarchus labrax* (Linné). *Ecotox. Environ. Safe.* **2011**, 74, 1896–1904. (doi:10.1016/j.ecoenv.2011.06.021)
- Bagi, A.; Riiser, E. S.; Molland, H. S.; Star, B.; Haverkamp, T. H. A.; Sydnes, M. O.; Pampanin, D. M. Gastrointestinal microbial community changes in Atlantic cod (*Gadus morhua*) exposed to crude oil. *BMC Microbiology* **2018**, 18, 25. (doi:10.1186/s12866-018-1171-2)
- Bagenal, T. B. Fish production in fresh waters; Blackwell, London, **1978**.

- Baini, M.; Martellini, T.; Cincinelli, A.; Campani, T.; Minutoli, R.; Panti, C.; ... Fossi, M. C. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. *Anal. Methods* **2017**, *9*, 1512–1520. (doi:10.1039/c6ay02674e)
- Balian, E. V.; Segers, H.; Lévêque, C.; Martens, K. The freshwater animal diversity assessment: an overview of the results. *Hydrobiologia* **2008**, *595*, 627–637. (doi:10.1007/s10750-007-9246-3)
- Barron, M. G.; Albro, P. W.; Hayton, W. L. Biotransformation of di (2-ethylhexyl) phthalate by rainbow trout. *Environ. Toxicol. Chem.* **1995**, *14*, 873-876. (doi:10.1002/etc.5620140519)
- Beaulieu, M.; Costantini, D. Biomarkers of oxidative status: missing tools in conservation physiology. *Conserv. Physiol.* **2014**, *2*, No. cou014. (doi:10.1093/conphys/cou014)
- Beaulieu, M.; Reichert, S.; Le Maho, Y.; Ancel, A.; Criscuolo, F. Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. *Funct. Ecol.* **2011**, *25*, 577-585. (doi:10.1111/j.1365-2435.2010.01825.x)
- Blanar, C. A.; Munkittrick, K. R.; Houlihan, J.; MacLachy, D. L.; Marcogliese, D. J. Pollution and parasitism in aquatic animals: A meta-analysis of effect size. *Aquat. Toxicol.* **2009**, *93*, 18–28. (doi:10.1016/j.aquatox.2009.03.002)
- Blévin, P.; Angelier, F.; Tartu, S.; Ruault, S.; Bustamante, P.; Herzke, D.; Moe, B.; Bech, C.; Gabrielsen, G. W.; Bustnes, J. O.; Chastel, O. Exposure to oxychlordan is associated with shorter telomeres in arctic breeding kittiwakes. *Sci. Total Environ.* **2016**, *563*, 125e130. (doi:10.1016/j.scitotenv.2016.04.096)
- Blévin, P.; Angelier, F.; Tartu, S.; Bustamante, P.; Herzke, D.; Moe, B.; Bech, C.; Wing Gabrielsen, G.; Bustnes, J. O.; Chastel, O. Perfluorinated substances and telomeres in an Arctic seabird: Cross-sectional and longitudinal approaches. *Environ. Poll.* **2017**, *230*, 360–367. (doi:10.1016/j.envpol.2017.06.060)
- Bolnick, D.; Snowberg, L.; Hirsch, P.; Lauber, C. L.; Org, E.; Parks, B.; Lusi, A. J.; Knight, R.; Caporaso, J. G.; Svanbäck, R. Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nat. Commun.* **2014**, *5*, 4500. (doi:10.1038/ncomms5500)
- Bols, N. C.; Brubacher, J. L.; Ganassin, R. C.; Lee, L. E. J. Ecotoxicology and innate immunity in fish. *Dev. Comp. Immunol.* **2001**, *21*, 856–873. (doi:10.1016/S0145-305X(01)00040-4)
- Boonekamp, J. J.; Bauch, C.; Mulder, E.; Verhulst, S. Does oxidative stress shorten telomeres? *Biol. Letters* **2017**, *13*, 20170164. (doi:10.1098/rsbl.2017.0164)
- Borkovcova, M.; Fiser, V.; Bednarova, M.; Havlicek, Z.; Adámková, A.; Mlcek, J.; Jurikova, T.; Balla, S.; Adámek, M. Effect of accumulation of heavy metals in the red fox intestine on the prevalence of its intestinal parasites. *Animals* **2020**, *10*, 343. (doi:10.3390/ani10020343)
- Brázová, T.; Hanzelová, V.; Miklisová, D. Bioaccumulation of six PCB indicator congeners in a heavily polluted water reservoir in Eastern Slovakia: tissue-specific distribution in fish and their parasites. *Parasitol. Res.* **2012**, *111*, 779–786. (doi:10.1007/s00436-012-2900-3)
- Britton, J. R.; Andreou, D. Parasitism as a driver of trophic niche specialisation. *Trends Parasitol.* **2016**, *32*, 437–445. (doi:10.1016/j.pt.2016.02.007)
- Brock, J. W.; Bell, J. M.; Guillette, L. J. Urinary phthalate metabolites in American alligators (*Alligator mississippiensis*) from selected Florida wetlands. *Arch. Environ. Con. Tox.* **2016**, *71*, 1–6. (doi:10.1007/s00244-015-0260-6)
- Brown, A.; Pascoe, D. Parasitism and host sensitivity to cadmium: an acanthocephalan infection of the freshwater amphipod *Gammarus pulex*. *J. Appl. Ecol.* **1989**, *26*, 473-487. (doi:10.2307/2404075)

- Bruslé, J.; Quignard J. P. Biologies des poissons d'eau douce européens. Lavoisier. Paris **2001**, p. 625.
- Bush, A. O.; Lafferty, K. D.; Lotz, J. M.; Shostak, A. W. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* **1997**, 83, 575–583. (doi:10.2307/3284227)
- Busst, G. M.; Britton, J. R. Precision of the age-length increments of three cyprinids: effects of fish number and sub-sampling strategy. *J. Fish Biol.* **2014**, 84, 1926–1939. (doi:10.1111/jfb.12409)
- Butt, R. L.; Volkoff, H. Gut microbiota and energy homeostasis in fish. *Front. Endocrinol.* **2019**, 10, 9. (doi:10.3389/fendo.2019.00009)
- Cabana, G.; Rasmussen, J. B. Model in good chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* **1994**, 372, 255–257.
- Caffrey, J. M., Acevedo, S., Gallagher, K., Britton, R. Chub (*Leuciscus cephalus*): a new potentially invasive fish species in Ireland. *Aquatic Invasions* **2008**, 3, 201–209. (doi:10.3391/ai.2008.3.2.11)
- Carré, C.; Meybeck, M.; Garnier, J.; Chong, N.; Deroubaix, J. F.; Flipo, N.; Goutte, A.; Le Pichon, C.; Seguin, L.; Tournebize, J. River Basin Visions: Tools and Approaches from Yesterday to Tomorrow. *The Handbook of Environmental Chemistry* **2020**. (doi:10.1007/698_2019_438)
- Carreras-Aubets, M.; Montero, F. E.; Kostadinova, A.; Carrasson, M. Parasite communities in the red mullet, *Mullus barbatus* L. respond to smallscale variation in the levels of polychlorinated biphenyls in the Western Mediterranean. *Mar. Pollut. Bull.* **2012**, 64, 1853–1860.
- Chatterjee, N. S.; Utture, S.; Banerjee, K.; Shabeer, A.; Kamble, N.; Mathew, S.; Kumar, K. A. Multiresidue analysis of multiclass pesticides and polyaromatic hydrocarbons in fatty fish by gas chromatography tandem mass spectrometry and evaluation of matrix effect. *Food. Chem.* **2016**, 196, 1–8.
- Claus, S. P.; Guillou, H.; Ellero-Simatos, S. The gut microbiota: a major player in the toxicity of environmental pollutants? *Npj Biofilms and Microbiomes* **2017**, 3, 17001. (doi:10.1038/npjbiofilms.2017.1)
- Clotfelter, E. D.; Lapidus, S. J. H.; Brown, A. C. The effects of temperature and dissolved oxygen on antioxidant defences and oxidative damage in the fathead minnow *Pimephales promelas*. *J. Fish Biol.* **2013**, 82, 1086–1092. (doi:10.1111/jfb.12050)
- Collinder, E.; Bjornhag, G.; Cardona, M.; Norin, E.; Rehbinder, C.; Midtvedt, T. Gastrointestinal host-microbial interactions in mammals and fish: comparative studies in man, mice, rats, pigs, horses, cows, elk, reindeer, salmon and cod. *Microb. Ecol. Health Dis.* **2003**, 15, 66–78. (doi:10.1080/08910600310014980)
- Collins, S. L.; Patterson, A. D. The gut microbiome: an orchestrator of xenobiotic metabolism. *Acta Pharm. Sin. B* **2019**, 10, 19–32. (doi:10.1016/j.apsb.2019.12.001)
- Cong, B.; Liu, C.; Wang, L.; Chai, Y. The impact on antioxidant enzyme activity and related gene expression following adult zebrafish (*Danio rerio*) exposure to dimethyl phthalate. *Animals* **2020**, 10, 717. (doi:10.3390/ani10040717)
- Coors, A.; De Meester, L. Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in *Daphnia magna*. *J. Appl. Ecol.* **2008**, 45, 1820–1828. (doi:10.1111/j.1365-2664.2008.01566.x)
- Cornet, S.; Franceschi, N.; Bauer, A.; Rigaud, T.; Moret, Y. Immune depression induced by acanthocephalan parasites in their intermediate crustacean host: Consequences for the risk of super-infection and links with host behavioural manipulation. *Int. J. Parasitol.* **2009**, 39, 221–229. (doi:10.1016/j.ijpara.2008.06.007)

- Costantini, D. Oxidative stress ecology and the d-ROMs test: facts, misfacts and an appraisal of a decade's work. *Behav. Ecol. Sociobiol.* **2016**, 70, 809–820. (doi:10.1007/s00265-016-2091-5)
- D'Adamo, R.; Pelosi, S.; Trotta, P.; Sansone, G. Bioaccumulation and biomagnification of polycyclic aromatic hydrocarbons in aquatic organisms. *Mar. Chem.* **1997**, 56, 45–49. (doi:10.1016/s0304-4203(96)00042-4)
- Dautremepuits, C.; Betoulle, S.; Vernet, G. Antioxidant response modulated by copper in healthy or parasitized carp (*Cyprinus carpio* L.) by *Ptychobothrium* sp. (Cestoda). *Biochim. Biophys. Acta* **2002**, 1673, 4–8. (doi:10.1016/s0304-4165(02)00328-8)
- Dautremepuits, C.; Betoulle, S.; Vernet, G. Stimulation of antioxidant enzymes levels in carp (*Cyprinus carpio* L.) infected by *Ptychobothrium* sp. (Cestoda). *Fish Shellfish Immunol.* **2003**, 15, 467–471. (doi:10.1016/s1050-4648(03)00007-x)
- Defo, M. A.; Gendron, A. D.; Head, J.; Pilote, M.; Turcotte, P.; Marcogliese, D. J.; Houde, M. Cumulative effects of cadmium and natural stressors (temperature and parasite infection) on molecular and biochemical responses of juvenile rainbow trout. *Aquat. Toxicol.* **2019**, 217, 105347. (doi:10.1016/j.aquatox.2019.105347)
- Dong, J.; Li, X.; Zhang, R.; Zhao, Y.; Wu, G.; Liu, J.; Zhu, X.; Li, L. Comparative analysis of the intestinal bacterial community and expression of gut immunity genes in the Chinese Mitten Crab (*Eriocheir sinensis*). *AMB Express* **2018**, 8, 192. (doi:10.1186/s13568-018-0722-0)
- Dragun, Z.; Tepić, N.; Krasnići, N.; Teskeredžić, E. Accumulation of metals relevant for agricultural contamination in gills of European chub (*Squalius cephalus*). *Environ. Sci. Pollut. R.* **2016**, 23, 16802–16815. (doi:10.1007/s11356-016-6830-y)
- Dromard, C. R.; Bouchon-Navaro, Y.; Cordonnier, S.; Guéné, M.; Harmelin-Vivien, M.; Bouchon, C. Different transfer pathways of an organochlorine pesticide across marine tropical food webs assessed with stable isotope analysis. *PLOS ONE* **2018**, 13, e0191335. (doi:10.1371/journal.pone.0191335)
- Du, S. N. N.; Choi, J. A.; McCallum, E. S.; McLean, A. R.; Borowiec, B. G.; Balshine, S.; Scott, G. R. Metabolic implications of exposure to wastewater effluent in bluegill sunfish. *Comp. Biochem. Phys. C* **2019**, 108562. (doi:10.1016/j.cbpc.2019.108562)
- Dudgeon D.; Arthington A. H.; Gessner M. O.; Kawabata Z. I.; Knowler D. J.; Lévêque C.; Naiman R. J.; Prieur-Richard A. H.; Soto D.; Stiassny M. L. J.; Sullivan C. A. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biol. Rev.* **2006**, 81; 163. (doi:10.1017/S1464793105006950)
- Dunier, M. Water pollution and immunosuppression of freshwater fish. *Ital. J. Zool.* **1996**, 63, 303–309. (doi:10.1080/11250009609356150)
- Dupuy, C.; Galland, C.; Devaux, A.; Bony, S.; Loizeau, V.; Danion, M.; Pichereau, V.; Fournier, M.; Laroche, J. Responses of the European flounder (*Platichthys flesus*) to a mixture of PAHs and PCBs in experimental conditions. *Environ. Sci. Pollut. R.* **2014**, 21, 13789–13803. (doi:10.1007/s11356-014-2563-y)
- Ellis, A. E. Lysozyme assays. *Technol. fish immunol.* **1990**, 1, 101–103.
- European Chemical Agency. **2017**. Online : <http://echa.europa.eu/fr/home>
- European Commission. Common Implementation Strategy for the Water Framework Directive (2000/60/EC): Guidance document no. 32 on biota monitoring (the implementation of EQS Biota) under the Water Framework Directive. Technical report, **2014**. Luxembourg.
- European Environment Agency. Chemicals in European waters: Knowledge developments. Technical report 18, **2018**. Copenhagen, Denmark.
- Evariste, L.; Barret, M.; Mottier, A.; Mouchet, F.; Gauthier, L.; Pinelli, E. Gut microbiota of aquatic organisms: A key endpoint for ecotoxicological studies. *Environ. Pollut.* **2019**, 248, 989–999. (doi:10.1016/j.envpol.2019.02.101)

- Everaarts, J. M.; Shugart, L. R.; Gustin, M. K.; Hawkins, W. E.; Walker, W. W. Biological markers in fish: DNA integrity, hematological parameters and liver somatic index. *Mar. Environ. Res.* **1993**, 35, 101–107. (doi:10.1016/0141-1136(93)90021-Q)
- Fatima, M.; Mandiki, S. N. M.; Douxfils, J.; Silvestre, F.; Coppe, P.; Kestemont, P. Combined effects of herbicides on biomarkers reflecting immune–endocrine interactions in goldfish: Immune and antioxidant effects. *Aquat. Toxicol.* **2007**, 81, 159–167. (doi:10.1016/j.aquatox.2006.11.013)
- Ferguson, K. K.; Loch-Caruso, R.; Meeker, J. D. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999–2006. *Environ. Res.* **2011**, 111, 718–726. (doi:10.1016/j.envres.2011.02.002)
- Filipović Marijić, V.; Vardić Smrzlić, I.; Raspor, B. Effect of acanthocephalan infection on metal, total protein and metallothionein concentrations in European chub from a Sava River section with low metal contamination. *Sci. Total Environ.* **2013**, 463–464, 772–780. (doi:10.1016/j.scitotenv.2013.06.041)
- Fisk, A. T.; Hobson, K. A.; Norstrom, R. J. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the North water Polynya marine food web. *Environ. Sci. Technol.* **2001**, 35, 732–738.
- Flammarion, P.; Migeon, B.; Garric, J. Statistical analysis of cyprinid ethoxyresorufin-O-deethylase data in a large French watershed. *Ecotox. Environ. Safe.* **1998**, 40, 144–153. (doi:10.1006/eesa.1998.1655)
- Förlin, L.; Haux, C. Sex differences in hepatic cytochrome P-450 monooxygenase activities in rainbow trout during an annual reproductive cycle. *J. Endocrinol.* **1990**, 124, 207–213.
- Fossi, M. C.; Panti, C.; Guerranti, C.; Coppola, D.; Giannetti, M.; Marsili, L.; Minutoli, R. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (*Balaenoptera physalus*). *Mar. Pollut. Bull.* **2012**, 64, 2374–2379. (doi:10.1016/j.marpolbul.2012.08.013)
- Fossi, M. C.; Coppola, D.; Bains, M.; Giannetti, M.; Guerranti, C.; Marsili, L.; ... Clò, S. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). *Mar. Environ. Res.* **2014**, 100, 17–24.
- Fourgous, C.; Chevreuil, M.; Alliot, F.; Amilhat, E.; Faliex, E.; Paris-Palacios, S.; Teil, M. J.; Goutte, A. Phthalate metabolites in the European eel (*Anguilla anguilla*) from Mediterranean coastal lagoons. *Sci. Total Environ.* **2016**, 569, 1053–1059. (doi:10.1016/j.scitotenv.2016.06.159)
- Foyosal, M. J.; Fotedar, R.; Tay, C.; Gupta, S. K. Dietary supplementation of black soldier fly (*Hermetica illucens*) meal modulates gut microbiota, innate immune response and health status of marron (*Cherax cainii*, Austin 2002) fed poultry-by-product and fishmeal based diets. *PeerJ* **2019**, 7, e6891. (doi:10.7717/peerj.6891)
- Frank, S. N.; Faust, S.; Kalbe, M.; Trubiroha, A.; Kloas, W.; Sures, B. Fish hepatic glutathione-S-transferase activity is affected by the cestode parasites *Schistocephalus solidus* and *Ligula intestinalis* : evidence from field and laboratory studies. *Parasitology* **2011**, 138, 939–944. (doi:10.1017/S003118201100045X)
- Franklin, M. R.; Yost, G. S. *Biotransformation: a balance between bioactivation and detoxification*. In: Williams, P.L.; James, R.C.; Roberts, S.M. (Eds.). Principles of Toxicology: Environmental and Industrial Applications. John Wiley & Sons, New York, NY **2000**, 57–86. (doi:10.1002/0471231800.ch3)
- Fredrich, F.; Ohmann, S.; Curio, B.; Kirschbaum, F. Spawning migrations of the chub in the River Spree, Germany. *J. Fish Biol.* **2003**, 63, 710–723. (doi:10.1046/j.1095-8649.2003.00184.x)

- Friesen, O. C.; Goellner, S.; Poulin, R.; Lagrue, C. Parasites shape community structure and dynamics in freshwater crustaceans. *Parasitology* **2019a**, 1–12. (doi:10.1017/s0031182019001483)
- Friesen, C. R.; de Graaf, S. P.; Olsson, M. The relationship of body condition, superoxide dismutase, and superoxide with sperm performance. *Behav. Ecol.* **2019b**, 32, 47. (doi:10.1093/beheco/arz086)
- Fu, P. P.; Xiong, F.; Feng, W. W.; Zou, H.; Wu, S. G.; Li, M.; Wang, G. T.; Li, W. X. Effect of intestinal tapeworms on the gut microbiota of the common carp, *Cyprinus carpio*. *Parasites Vectors* **2019**, 12, 252. (doi:10.1186/s13071-019-3510-z)
- Gagné, F. Biochemical Ecotoxicology: Principles and Methods. Elsevier, London, **2014**.
- Galli, P.; Crosa, G.; Occhipinti Ambrogi, A. Heavy metals concentrations in acanthocephalans parasites compared to their fish host. *Chemosphere* **1998**, 37, 2983–2988. (doi:10.1016/S0045-6535(98)00340-3)
- Gaulke, C. A.; Barton, C. L.; Proffitt, S.; Tanguay, R. L.; Sharpton, T. J. Triclosan exposure is associated with rapid restructuring of the microbiome in adult zebrafish. *PLoS One* **2016**, 11, e0154632. (doi:10.1371/journal.pone.0154632)
- Gaulke, C. A.; Martins, M. L.; Watral, V. G.; Humphreys, I. R.; Spagnoli, S. T.; Kent, M. L.; Sharpton, T. J. A longitudinal assessment of host-microbe-parasite interactions resolves the zebrafish gut microbiome's link to *Pseudocapillaria tomentosa* infection and pathology. *Microbiome* **2019**, 7, 10. (doi:10.1186/s40168-019-0622-9)
- Geiger, S.; Le Vaillant, M.; Lebard, T.; Reichert, S.; Stier, A.; Le Maho, Y.; Criscuolo, F. Catching-up but telomere loss: half-opening the black box of growth and ageing trade-off in wild king penguin chicks. *Mol. Ecol.* **2012**, 21, 1500-1510. (doi :10.1111/j.1365-294x.2011.05331.x)
- Gerhardt, A.; Bloor, M.; Mills, C. L. Gammarus: Important taxon in freshwater and marine changing environments. *Int. J. Zool.* **2011**, 2011, 1–2 ID 524276.
- Gilbert, B. M.; Avenant-Oldewage, A. Hatchability and survival of oncomiracidia of *Paradiplozoon ichthyoxanthon* (Monogenea: Diplozoidae) exposed to aqueous aluminium. *Parasites Vectors* **2016**, 9, 420. (doi:10.1186/s13071-016-1706-z)
- Gismondi, E.; Cossu-Leguille, C.; Beisel, J. N. Acanthocephalan parasites: Help or burden in gammarid amphipods exposed to cadmium? *Ecotoxicology* **2012**, 21, 1188–1193. (doi:10.1007/s10646-012-0873-8)
- Goater, T. M.; Goater, C. P.; Esch, G. W. *Parasitism: The Diversity and Ecology of Animal Parasites*, 2nd edn. Cambridge, Cambridge University Press, **2014**.
- Gobas, F. A. P. C.; Kelly, B. C.; Arnot, J. A. Quantitative structure activity relationships for predicting the bioaccumulation of POPs in terrestrial food-webs. *Quant. Struct-Act Relat. Comb. Sci.* **2003**, 22, 329–336.
- Goutte, A.; Alliot, F.; Budzinski, H.; Simonnet-Laprade, C.; Santos, R.; Lachaux, V., Labadie, P. Trophic transfer of micropollutants and their metabolites in an urban riverine food web. *Environ. Sci. Technol* **2020**, 54, 8043–8050. (doi:10.1021/acs.est.0c01411)
- Grunst, A. S.; Grunst, M. L.; Bervoets, L.; Pinxten, R.; Eens, M. Proximity to roads, but not exposure to metal pollution, is associated with accelerated developmental telomere shortening in nestling great tits. *Environ. Poll.* **2020**, 256, 113373.
- Guardiola, F. A.; Cuesta, A.; Abellán, E.; Meseguer, J.; Esteban, M. A. Comparative analysis of the humoral immunity of skin mucus from several marine teleost fish. *Fish Shellfish Immunol.* **2014**, 40, 24–31. (doi:10.1016/j.fsi.2014.06.018)
- Hamer, H.; Jonkers, D.; Troost, F. J.; Bast, A.; Vanhoutvin, S.; Venema, K.; Brummer, R. J. Butyrate modulates oxidative stress in the colonic mucosa of healthy humans. *Gastroenterology* **2007**, 132, A79. (doi:10.1016/j.clnu.2008.11.002)

- Hanache, P.; Spataro, T.; Firmat, C.; Boyer, N.; Fonseca, P.; Médoc, V. Noise-induced reduction in the attack rate of a planktivorous freshwater fish revealed by functional response analysis. *Freshwater Biol.* **2020**, *65*, 75–85. (doi:10.1111/fwb.13271)
- Harried, B. L.; Daugherty, D. J.; Hoeinghaus, D. J.; Roberts, A. P.; Venables, B. J.; Sutton, T. M.; Soulen, B. K. Population contributions of large females may be eroded by contaminant body burden and maternal transfer: a case study of alligator gar. *N. Am. J. Fish. Manage.* **2020**, *40*, 566–579. (doi:10.1002/nafm.10382)
- Hatakeyama, H.; Yamazaki, H.; Nakamura, K. I.; Izumiya-Shimomura, N.; Aida, J.; Suzuki, H.; Tsuchida, S.; Matsuura, M.; Takubo, K.; Ishikawa, N. Telomere attrition and restoration in the normal teleost *Oryzias latipes* are linked to growth rate and telomerase activity at each life stage. *Aging* **2016**, *8*, 62–75. (doi:10.18632/aging.100873)
- Hebert, C. E.; Keenleyside, K. A. To normalize or not to normalize? Fat is the question. *Environ. Toxicol. Chem.* **1995**, *14*, 801–807.
- Hecker, M.; Karbe, L. Parasitism in fish—an endocrine modulator of ecological relevance? *Aquat. Toxicol.* **2005**, *72*, 195–207. (doi:10.1016/j.aquatox.2004.12.008)
- Hellawell, J. M. The autecology of the chub, *Squalius cephalus* (L.), of the River Lugg and the Afon Llynfi. *Freshwater Biol.* **1971**, *1*, 369–387. (doi:10.1111/j.1365-2427.1971.tb01569.x)
- Henríquez-Hernández, L. A.; Carretón, E.; Camacho, M.; Montoya-Alonso, J. A.; Boada, L. D.; Valerón, P. F.; Córdón, Y. F.; Almeida-González, M.; Zumbado, M.; Luzardo, O. P. Influence of parasitism in dogs on their serum levels of persistent organochlorine compounds and polycyclic aromatic hydrocarbons. *Sci. Total Environ.* **2016**, *562*, 128–135. (doi: 10.1016/j.scitotenv.2016.03.204)
- Henríquez-Hernández, L. A.; Carretón, E.; Camacho, M.; Montoya-Alonso, J. A.; Boada, L. D.; Valerón, P. F.; Falcón-Cordón, Y.; Falcón-Cordón, S.; Almeida-González, M.; Zumbado, M.; Luzardo, O. P. The heartworm (*Dirofilaria immitis*) seems to be able to metabolize organochlorine pesticides and polychlorinated biphenyls: A case-control study in dogs. *Sci. Total Environ.* **2017**, *575*, 1445–1452.
- Hermabessiere, L.; Dehaut, A.; Paul-Pont, I.; Lacroix, C.; Jezequel, R.; Soudant, P.; Duflos, G. Occurrence and effects of plastic additives on marine environments and organisms: A review. *Chemosphere* **2017**, *182*, 781–793. (doi:10.1016/j.chemosphere.2017.05.096)
- Hewitt, S.; Fenet, H.; Casellas, C. Induction of erod activity in european Eel (*Anguilla anguilla*) by different polychlorobiphenyls (PCBs). *Water Sci. Technol.* **1998**, *38*, 245–252. (doi:10.1016/s0273-1223(98)00625-8)
- HIS Markit. Plasticizers, **2018**. Online: <https://ihsmarkit.com/products/plasticizers-chemical-economics-handbook.html>
- Hoole, D.; Lewis, J. W.; Schuwerack, P. M. M.; Chakravarthy, C.; Shrive, A. K.; Greenhough, T. J.; Cartwright, J. R. Inflammatory interactions in fish exposed to pollutants and parasites: a role for apoptosis and C reactive protein. *Parasitology*, **2003**, *126*, S71–S85. (doi:10.1017/S003118200300377)
- Hursky, O.; Pietrock, M. Intestinal nematodes affect selenium bioaccumulation, oxidative stress biomarkers, and health parameters in juvenile rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* **2015**, *49*, 2469–2476. (doi:10.1021/es5048792)
- Ibáñez-Álamo, J. D.; Pineda-Pampliega, J.; Thomson, R. L.; Aguirre, J. I.; Díez-Fernández, A.; Faivre, B.; Verhulst, S. Urban blackbirds have shorter telomeres. *Biol. Lett.* **2018**, *14*, 20180083. (doi:10.1098/rsbl.2018.0083)
- Jackson, A. L.; Inger, R.; Parnell, A. C.; Bearhop, S. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *J. Anim. Ecol.* **2011**, *80*, 595–602. (doi:10.1111/j.1365-2656.2011.01806.x)

- Jacobson, K. C.; Arkoosh, M. R.; Kagley, A. N.; Clemons, E. R.; Collier, T. K.; Casillas, E. Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile chinook salmon. *J. Aquat. Anim. Health* **2003**, *15*, 1–12. (doi:10.1577/1548-8667(2003)015<0001:ceonaa>2.0.co;2)
- Jankovská, I.; Miholová, D.; Bejček, V.; Vadlejch, J.; Šulc, M.; Száková, J.; Langrová, I. Influence of parasitism on trace element contents in tissues of red fox (*Vulpes vulpes*) and its parasites *Mesocestoides* spp. (Cestoda) and *Toxascaris leonina* (Nematoda). *Arch. Environ. Contam. Toxicol.* **2010**, *58*, 469–477.
- Jasinska, E. J.; Goss, G. G.; Gillis, P. L.; Van Der Kraak, G. J.; Matsumoto, J.; de Souza Machado, A. A.; Giacomini, M.; Moon, T. W.; Massarsky, A.; Gagné, F.; Servos, M. R.; Wilson, J.; Sultana, T.; Metcalfe, C. D. Assessment of biomarkers for contaminants of emerging concern on aquatic organisms downstream of a municipal wastewater discharge. *Sci Total Environ.* **2014**, *530–531*, 140–153. (doi:10.1016/j.scitotenv.2015.05.080)
- Jaskelioff, M.; Muller, F. L.; Paik, J. H.; Thomas, E.; Jiang, S.; Adams, A. C.; Sahin, E.; Kost-Alimova, M.; Protopopov, A.; Cadiñanos, J.; Horner, J. W.; Maratos-Flier, E.; Depinho, R. A. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature* **2011**, *469*, 102–106. (doi:10.1038/nature09603)
- Jonsson, N.; Jonsson, B. Energy allocation among developmental stages, age groups, and types of Atlantic salmon (*Salmo salar*) spawners. *Can. J. Fish. Aquat. Sci.* **2003**, *60*, 506–516. (doi:10.1139/f03-042)
- Kampire, E.; Rubidge, G.; Adams, J. B. Distribution of polychlorinated biphenyl residues in several tissues of fish from the North End Lake, Port Elizabeth, South Africa. *Water SA* **2015**, *41*, 559–570. (doi:10.4314/wsa.v41i4.16)
- Karell, P.; Bensch, S.; Ahola, K.; Asghar, M. Pale and dark morphs of tawny owls show different patterns of telomere dynamics in relation to disease status. *P. Roy. Soc. B-Biol. Sci.* **2017**, *284*, 20171127. (doi:10.1098/rspb.2017.1127)
- Kasumyan, A. O. Effects of chemical pollutants on foraging behavior and sensitivity of fish to food stimuli. *J. Ichthyol.* **2001**, *41*, 76–87.
- Kelly, B. C.; Ikonomidou, M. G.; Blair, J. D.; Morin, A. E.; Gobas, F. A. Food web-specific biomagnification of persistent organic pollutants. *Science* **2007**, *317*, 236–239. (doi:10.1126/science.1138275)[X] Pierce, J. J.; Weiner, R. F.; Vesilind, P. A. *Water pollution*. In *Environmental pollution and control - 4th ed.* Butterworth-Heinemann (eds), **1998**.
- Kennedy, C. R. *Ecology of the Acanthocephala*. Cambridge University Press, New York, New York, 249 p, **2006**.
- Kerambrun, E.; Henry, F.; Cornille, V.; Courcot, L.; Amara, R. A combined measurement of metal bioaccumulation and condition indices in juvenile European flounder, *Platichthys flesus*, from European estuaries. *Chemosphere* **2013**, *91*, 498–505. (doi:10.1016/j.chemosphere.2012.12.010)
- Kime, D. E. The effects of pollution on reproduction in fish. *Rev. Fish Biol. Fisher.* **1995**, *5*, 52–95. (doi:10.1007/bf01103366)
- Ko, F. C.; Pan, W. L.; Cheng, J. O.; Chen, T. H.; Kuo, F. W.; Kao, S. J.; Chang, C. W.; Ho, H. C.; Wang, W. H.; Fang, L. S. Persistent organic pollutants in Antarctic notothenioid fish and invertebrates associated with trophic levels. *PLOS One* **2018**, *13*:e0194147. (doi:10.1371/journal.pone.0194147)
- Köhler, P.; Voigt, W. P. *Nutrition and metabolism*. In: Mehlhorn, H. (Ed.), *Parasitology in Focus: Facts and Trends*. Springer-Verlag, Berlin, **1988**, pp. 412–453.
- Labadie, P.; Alliot, F.; Bourges, C.; Desportes, A.; Chevreuil, M. Determination of polybrominated diphenyl ethers in fish tissues by matrix solid phase dispersion and gas

- chromatography coupled to triple quadrupole mass spectrometry: Case study on European eel (*Anguilla anguilla*) from Mediterranean coastal lagoons. *Anal Chim Acta* **2010**, 675, 97–105. (doi:10.1016/j.aca.2010.07.010)
- Lacaze, É.; Gendron, A. D.; Miller, J. L.; Colson, T.-L. L.; Sherry, J. P.; Giraudo, M.; Marcogliese, D. J.; Houde, M. Cumulative effects of municipal effluent and parasite infection in yellow perch: A field study using high-throughput RNA-sequencing. *Sci. Total Environ.* **2019**, 665, 797–809. (doi:10.1016/j.scitotenv.2019.02.155)
- Lafferty, K. D. Environmental parasitology: what can parasitology tell us about human impacts on the environment? *Parasitol. Today* **1997**, 13, 251–255.
- Lagrue, C.; Poulin, R. Measuring fish body condition with or without parasites: does it matter? *J. Fish Biol.* **2015**, 87, 836–847. (doi:10.1111/jfb.12749)
- Larno, V.; Laroche, J.; Launey, S.; Flammarion, P.; Devaux, A. Responses of chub (*Leuciscus cephalus*) populations to chemical stress, assessed by genetic markers, DNA damage and cytochrome P4501A induction. *Ecotoxicology* **2001**, 10, 145–158. doi: (10.1023/a:1016637809483)
- Le, T. T. Y.; Nachev, M.; Grabner, D.; Hendriks, A. J.; Sures, B. Development and validation of a biodynamic model for mechanistically predicting metal accumulation in fish-parasite systems. *PLoS ONE* **2016**, 11, e0161091. (doi:10.1371/journal.pone.0161091)
- Le Guernic, A.; Sanchez, W.; Palluel, O.; Bado-Nilles, A.; Floriani, M.; Turies, C.; Chadili, E.; Della Vedova, C.; Cavalié, I.; Adam-Guillermin, C.; Porcher, J. M.; Geffard, A.; Betoulle, S.; Gagnaire, B. Acclimation capacity of the three-spined stickleback (*Gasterosteus aculeatus*, L.) to a sudden biological stress following a polymetallic exposure. *Ecotoxicology* **2016**, 25, 1478–1499. (doi:10.1007/s10646-016-1699-6)
- Lenhardt, M.; Jarić, I.; Cvijanović, G.; Gačić, Z. Seasonal changes in condition, hepatosomatic index and parasitism in starlet (*Acipenser ruthenus* L.). *Turk. J. Vet. Anim. Sci.* **2009**; 33, 209–214. (doi:10.3906/vet-0710-14)
- Leung, J. M.; Graham, A. L.; Knowles, S. C. L. Parasite-microbiota interactions with the vertebrate gut: synthesis through an ecological lens. *Front. Microbiol.* **2018**, 9, 843. (doi:10.3389/fmicb.2018.00843)
- Li, H.; Jia, W. Cometabolism of microbes and host: implications for drug metabolism and drug-induced toxicity. *Nature* **2013**, 94, 1–8. (doi:10.1038/clpt.2013.157)
- Li, X. F.; Tian, H. Y.; Zhang, D. D.; Jiang, G. Z.; Liu, W. B. Feeding frequency affects stress, innate immunity and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*. *Fish Shellfish Immunol.* **2014**, 38, 80–87.
- Ling, F.; Steinel, N.; Weber, J.; Ma, L.; Smith, C.; Correa, D.; Zhu, B.; Bolnick, D.; Wang, G. The gut microbiota response to helminth infection depends on host sex and genotype. *ISME J.* **2020**, 14, 1141–1153. (doi:10.1038/s41396-020-0589-3)
- Livingstone, D. R. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comp. Biochem. Phys. A* **1998**, 120, 43–49. (doi:10.1016/S1095-6433(98)10008-9)
- Louzon, M.; Coeurdassier, M.; Gimbert, F.; Pauget, B.; de Vauflery, A. Telomere dynamic in humans and animals: Review and perspectives in environmental toxicology. *Environ Int.* **2019**, 131, 105025. (doi:10.1016/j.envint.2019.105025)
- Maceda-Veiga, A.; Green, A. J.; Poulin, R.; Lagrue, C. Body Condition Peaks at Intermediate Parasite Loads in the Common Bully *Gobiomorphus cotidianus*. *PLOS One* **2016**, 11, e0168992. (doi:10.1371/journal.pone.0168992)
- Mackintosh, C. E.; Maldonado, J.; Hongwu, J.; Hoover, N.; Chong, A.; Ikononou, M. G.; Gobas, F. A. P. C. Distribution of phthalate esters in a marine aquatic food web: comparison to polychlorinated biphenyls. *Environ. Sci. Technol.* **2004**, 38, 2011–2020. (doi:10.1021/es034745r)

- Madenjian, C. P.; Stevens, A. L.; Stapanian, M. A.; Batterman, S. A.; Chernyak, S. M.; Menczer, J. E.; McIntyre, P. B. Sex difference in PCB Concentrations of a Catostomid Fish. *J. Environ. Anal. T.* **2017**, 07. (doi:10.4172/2161-0525.1000515)
- Malaj, E.; von der Ohe, P. C.; Grote, M.; Kühne, R.; Mondy, C. P.; Usseglio-Polatera, P.; Brack, W.; Schäfer, R. B. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, 111, 9549–9554. (doi:10.1073/pnas.1321082111)
- Malek, M.; Haseli, M.; Mobedi, I.; Ganjali, M. R.; MacKenzie, K. Parasites as heavy metal bioindicators in the shark *Carcharhinus dussumieri* from the Persian Gulf. *Parasitology* **2007**, 134, 1053. (doi:10.1017/S0031182007002508)
- Marcogliese, D.; Brambilla, L.; Gagné, F.; Gendron, A. Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*. *Dis. Aquat. Organ.* **2005**, 63, 77–84. (doi:10.3354/dao063077)
- Marcogliese, D. J.; Pietrock, M. Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends Parasitol.* **2011**, 27, 123–30. (doi:10.1016/j.pt.2010.11.002)
- Martin, B. T.; Heintz, R.; Danner, E. M.; Nisbet, R. M. Integrating lipid storage into general representations of fish energetics. *J. Anim. Ecol.* **2017**, 86, 812–825. (doi:10.1111/1365-2656.12667)
- Martínez-Álvarez, R. M.; Morales, A. E.; Sanz, A. antioxidant defenses in fish: biotic and abiotic factors. *Rev. Fish Biol. Fisher.* **2005**, 15, 75–88. (doi:10.1007/s11160-005-7846-4)
- Matei, A. V.; Farcas, A.; Florian, C.; Florescu, M.; Coman, G. *Pollutants Biotransformation*. In: Simeonov L., Macaev F., Simeonova B. (eds) Environmental security assessment and management of obsolete pesticides in Southeast Europe. NATO Science for Peace and Security Series C: Environmental Security. Springer, Dordrecht, **2013**. (doi:10.1007/978-94-007-6461-3_9)
- Mazéas, O.; Budzinski, H. Solid phase extraction for the quantification of polycyclic aromatic hydrocarbon metabolites in biological fluids. *Anal Bioanal Chem.* **2005**, 383, 985–990.
- McCord, J. M.; Fridovich, I. Superoxide dismutase: an enzymic function for erythrocuprein (hemocuprein). *J Biol Chem.* **1969**, 244, 6049–6055.
- McLaughlin, M. J.; Lanno, R. Use of “Bioavailability” as a term in ecotoxicology. *Integr. Environ. Asses.* **2013**, 10, 138–140. (doi:10.1002/ieam.1497)
- McLennan, D.; Recknagel, H.; Elmer, K. R.; Monaghan, P. Distinct telomere differences within a reproductively bimodal common lizard population. *Funct. Ecol.* **2019**, 33, 1917–1927. (doi:10.1111/1365-2435.13408)
- Meillere, A.; Brischoux, F.; Ribout, C.; Angelier, F. Traffic noise exposure affects telomere length in nestling house sparrows. *Biol. Lett.* **2015**, 11, 1–5. (doi:10.1098/rsbl.2015.0559)
- Merciai, R.; Guasch, H.; Kumar, A.; Sabater, S.; García-Berthou, E. Trace metal concentration and fish size: Variation among fish species in a Mediterranean river. *Ecotox. Environ. Safe.* **2014**, 107, 154–161. (doi:10.1016/j.ecoenv.2014.05.006)
- Michl, S. C.; Ratten, J. M.; Beyer, M.; Hasler, M.; LaRoche, J.; Schulz, C. The malleable gut microbiome of juvenile rainbow trout (*Oncorhynchus mykiss*): Diet-dependent shifts of bacterial community structures. *PLoS One* **2017**, 12, e0177735. (doi:10.1371/journal.pone.0177735)
- Midha, A.; Schlosser, J.; Hartmann, S. Reciprocal interactions between nematodes and their microbial environments. *Front. Cell. Infect. Mi.* **2017**, 7, 144. (doi:10.3389/fcimb.2017.00144)

- Milinski, M. Parasites determine a predator's optimal feeding strategy. *Behav. Ecol. Sociobiol.* **1984**, 15, 35–37.
- Mille, T.; Soulier, L.; Caill-Milly, N.; Cresson, P.; Morandau, G.; Monperrus, M. Differential micropollutants bioaccumulation in European hake and their parasites *Anisakis sp.* *Environ. Poll.* **2020**, 115021. (doi:10.1016/j.envpol.2020.115021)
- Morassutti, A. L.; Pinto, P. M.; Dutra, B. K.; Oliveira, G.T.; Ferreira, H. B.; Graeff-Teixeira, C. Detection of anti-oxidant enzymatic activities and purification of glutathione transferases from *Angiostrongylus cantonensis*. *Exp. Parasitol.* **2011**, 127, 365–369. (doi:10.1016/j.exppara.2010.08.018)
- Morrill, A.; Provencher, J. F.; Gilchrist, H. G.; Mallory, M. L.; Forbes, M. R. Anti-parasite treatment results in decreased estimated survival with increasing lead (Pb) levels in the common eider *Somateria mollissima*. *P. Roy. Soc. B-Biol. Sci.* **2019**, 286, 1–9. (doi:10.1098/rspb.2019.1356)
- Morley, N. J.; Lewis, J. W.; Hoole, D. Pollutant-induced effects on immunological and physiological interactions in aquatic host-trematode systems: implications for parasite transmission. *J Helminthol.* **2006**, 80, 137–49. (doi:10.1079/JOH2006345)
- Nachev, M.; Sures, B. Seasonal profile of metal accumulation in the acanthocephalan *Pomphorhynchus laevis*: a valuable tool to study infection dynamics and implications for metal monitoring. *Parasites Vectors* **2016**, 9, 300. (doi:10.1186/s13071-016-1576-4)
- Nagyová, S.; Tölgyessy, P. Validation including uncertainty estimation of a GC-MS/MS method for determination of selected halogenated priority substances in fish using rapid and efficient lipid removing sample preparation. *Foods* **2019**, 8, 101. (doi:10.3390/foods8030101)
- Newbold, L. K.; Burthe, S. J.; Oliver, A. E.; Gweon, H. S.; Barnes, C. J.; Daunt, F.; van der Gast, C. J. Helminth burden and ecological factors associated with alterations in wild host gastrointestinal microbiota. *ISME J.* **2016**, 11, 663–675. (doi:10.1038/ismej.2016.153)
- OECD. Emission scenario document on plastic additives. Series on emission scenario documents, no. 3. Paris: Environmental directorate, OECD Environmental Health and Safety Publications, **2004**. Available on: <http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=ENV/JM/MONO%282004%298/REV1>,
- Ohiozebau, E.; Tandler, B.; Codling, G.; Kelly, E.; Giesy, J. P.; Jones, P. D. Potential health risks posed by polycyclic aromatic hydrocarbons in muscle tissues of fishes from the Athabasca and Slave Rivers, Canada. *Environ. Geochem. Hlth.* **2016**, 39, 139–160. (doi:10.1007/s10653-016-9815-3)
- Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid per-oxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, 95, 351–358.
- Olaisen, M.; Flatberg, A.; Granlund, A. V. B.; Røyset, E. S.; Martinsen, T. C.; Sandvik, A. K.; Fossmark, R. Bacterial mucosa-associated microbiome in inflamed and proximal noninflamed ileum of patients with Crohn's disease. *Inflamm. Bowel Dis.* **2020**, 26, 1000. (doi.org/10.1093/ibd/izaa107)
- Oluoch-Otiego, J.; Oyoo-Okoth, E.; Kiptoo, K. K. G.; Chemoiwa, E. J.; Ngugi, C. C.; Simiyu, G.; Omutange, E. S.; Ngure, V.; Opiyo, M. A. PCBs in fish and their cestode parasites in Lake Victoria. *Environ. Monit. Assess.* **2016**, 188, 483. (doi:10.1007/s10661-016-5483-0)
- Paul, M. J.; Meyer, J. L. Streams in the urban landscape. *Annu. Rev. Ecol. Syst.* **2001**, 32, 333–365. (doi:10.1146/annurev.ecolsys.32.081501.114040)

- Pegg, J.; Andreou, D.; Williams, C. F.; Britton, J. R. Consistent patterns of trophic niche specialization in host populations infected with a non-native copepod parasite. *Parasitology* **2017**, 144, 945–953. (doi:10.1017/s0031182017000075)
- Penczak, T. Movement pattern and growth ratio of tagged fish in two lowland rivers of central Poland. *Pol. J. Ecol.* **2006**, 54, 267–282.
- Petitjean, Q.; Jean, S.; Côte, J.; Larcher, T.; Angelier, F.; Ribout, C.; Perrault, A.; Laffaille, P.; Jacquin, L. Direct and indirect effects of multiple environmental stressors on fish health in human-altered rivers. *Sci. Total Environ.* **2020**, 140657. (doi:10.1016/j.scitotenv.2020.140657)
- Persson, M. E.; Larsson, P.; Stenroth, P. Biomagnification and polychlorinated biphenyl congener distribution in an aquatic predator-prey, host-parasite system. *Environ. Toxicol. Chem.* **2007**, 26, 837–843. (doi:10.1897/06-305R.1)
- Pierce, J. J.; Weiner, R. F.; Vesilind, P. A. *Water pollution*. In: Environmental pollution and control, 4th ed.; Butterworth-Heinemann, **1998**.
- Poulin, R. Toxic pollution and parasitism in freshwater fish. *Parasitol. Today* **1992**, 8, 58–61.
- Qiu, Z.; Yang, H.; Rong, L.; Ding, W.; Chen, J.; Zhong, L. Targeted Metagenome Based Analyses Show Gut Microbial Diversity of Inflammatory Bowel Disease patients. *Indian J. Microbiol.* **2017**, 57, 307–315. (doi:10.1007/s12088-017-0652-6)
- Quade, M. J.; Roth, J. A. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Vet. Immunol. Immunopathol.* **1997**, 58, 239–248. (doi:10.1016/S0165-2427(97)00048-2)
- Qu, R.; Feng, M.; Sun, P.; Wang, Z. A comparative study on antioxidant status combined with integrated biomarker response in *Carassius auratus* fish exposed to nine phthalates. *Environ. Toxicol.* **2015**, 30, 1125–1134.
- Radovanović, T. B.; Prokić, M. D.; Gavrić, J. P.; Despotović, S. G.; Gavrilović, B. R.; Borković-Mitić, S. S.; Pavlović, S. Z.; Saičić, Z. S. Glutathione-dependent enzyme activities and concentrations of glutathione, vitamin E and sulfhydryl groups in barbel (*Barbus barbus*) and its intestinal parasite *Pomphorhynchus laevis* (Acanthocephala). *Ecol. Indic.* **2015**, 54, 31–38. (doi:10.1016/j.ecolind.2015.02.005)
- Raikova-Petrova, G.; Hamwi, N.; Petrov, I. Spawning, sex ratio and relationship between fecundity, length, weight and age of chub (*Squalius cephalus* L., 1758) in the middle stream of Iskar river (Bulgaria). *Acta Zool. Bulg.* **2012**, 64, 191–197.
- Ranta, E. Schistocephalus infestation improves prey-size selection by 3-spined sticklebacks, *Gasterosteus aculeatus*. *J. Fish Biol.* **1995**, 46, 156–158.
- Reichert, S.; Stier, A.; Zahn, S.; Arrivé, M.; Bize, P.; Massemin, S.; Criscuolo, F. Increased brood size leads to persistent eroded telomeres. *Front. Ecol. Evol.* **2014**, 2, 9. (doi:10.3389/fevo.2014.00009)
- Reichert, S.; Stier, A. Does oxidative stress shorten telomeres in vivo? A review. *Biol. Lett.* **2017**, 13, 20170463. (doi:10.1098/rsbl.2017.0463)
- Ren, J.; Wang, X.; Wang, C.; Gong, P.; Wang, X.; Yao, T. Biomagnification of persistent organic pollutants along a high-altitude aquatic food chain in the Tibetan Plateau: Processes and mechanisms. *Environ. Poll.* **2017**, 220, 636–643. (doi:10.1016/j.envpol.2016.10.019)
- Rian, M. B.; Vike-Jonas, K.; Gonzalez, S. V.; Ciesielski, T. M.; Venkatraman, V.; Lindstrøm, U.; ... Asimakopoulos, A. G. Phthalate metabolites in harbor porpoises (*Phocoena phocoena*) from Norwegian coastal waters. *Environ. Int.* **2020**, 137, 105525. (doi:10.1016/j.envint.2020.105525)
- Rinninella, E.; Raoul, P.; Cintoni, M.; Franceschi, F.; Miggianno, G.; Gasbarrini, A.; Mele, M. What is the healthy gut microbiota composition? A changing ecosystem across age,

- environment, diet, and diseases. *Microorganisms* **2019**, *7*, 14. (doi:10.3390/microorganisms7010014)
- Robin, D. C.; Marchand, P. A. Evolution of the biocontrol active substances in the framework of the European Pesticide Regulation (EC) No. 1107/2009. *Pest. Manag. Sci.* **2018**, *75*, 950–958. (doi:10.1002/ps.5199)
- Robinson, M. L.; Gomez-Raya, L.; Rauw, W. M.; Peacock, M. M. Fulton's body condition factor K correlates with survival time in a thermal challenge experiment in juvenile Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*). *J. Therm. Biol.* **2008**, *33*, 363–368. (doi:10.1016/j.jtherbio.2008.05.004)
- Roon, S. R.; Alexander, J. D.; Jacobson, K. C.; Bartholomew, J. L. Effect of Nanophyetus salmincola and bacterial co-infection on mortality of juvenile Chinook Salmon. *J. Aquat. Anim. Health* **2015**, *27*, 209–216. (doi:10.1080/08997659.2015.1094150)
- Ros, O.; Izaguirre, J. K.; Olivares, M.; Bizarro, C.; Ortiz-Zarragoitia, M.; Cajaraville, M. P.; ... Vallejo, A. Determination of endocrine disrupting compounds and their metabolites in fish bile. *Sci. Total Environ.* **2015**, *536*, 261–267. (doi:10.1016/j.scitotenv.2015.07.074)
- Ros, O.; Vallejo, A.; Olivares, M.; Etxebarria, N.; Prieto, A. Determination of endocrine disrupting compounds in fish liver, brain, and muscle using focused ultrasound solid–liquid extraction and dispersive solid phase extraction as clean-up strategy. *Anal. Bioanal. Chem.* **2016**, *408*, 5689–5700. (doi:10.1007/s00216-016-9697-3)
- Ruus, A.; Skaare, J. U.; Ingebrigtsen, K. Accumulation of the lipophilic environmental contaminant lindane in metacercariae of *Bucephaloides gracilescens* (trematoda, bucephalidae) in the central nervous system of bullrout *Myoxocephalus scorpius*. *Dis. Aquat. Org.* **2001**, *48*, 75–77.
- Russell, R. W.; Gobas, F. A.; Haffner, G. D. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. *Environ. Toxicol. Chem.* **1999**, *18*, 1250–1257.
- Sakthivel, A.; Gopalakrishnan, A.; Selvakumar, P. Pathological manifestation of the Acanthocephalus dirus in *Thunnus albacares*. *Asian Pac. J. Trop. Dis.* **2016**, *6*, 350–353. (doi:10.1016/S2222-1808(15)61045-3)
- Salmón, P.; Nilsson, J. F.; Nord, A.; Bensch, S.; Isaksson, C. Urban environment shortens telomere length in nestling great tits, *Parus major*. *Biol. Lett.* **2016**, *12*, 20160155. (doi:10.1098/rsbl.2016.0155)
- Sanchez-Ramírez, C.; Vidal-Martinez, V. M.; Aguirre-Macedo, L.; Rodriguez-Canul, R. P.; Ceja-Moreno, V.; Gold-Bouchot, G.; Sures, B. *Cichlidogyrus sclerosus* (Monogenea: Ancyrocephalinae) and its host the Nile tilapia (*Oreochromis niloticus*) as bioindicators of chemical pollution. *J. Parasitol.* **2007**, *93*, 1097–1106. (doi:10.1645/ge-1162r.1)
- Sánchez, M. I.; Pons, I.; Martínez-Haro, M.; Taggart, M. A.; Lenormand, T.; Green, A. J. When parasites are good for health: cestode parasitism increases resistance to arsenic in brine shrimps. *PLoS Pathog.* **2016**, *12*, No. e1005459. (doi:10.1371/journal.ppat.1005459)
- Sánchez, C. A.; Becker, D. J.; Teitelbaum, C. S.; Barriga, P.; Brown, L. M.; Majewska, A. A.; Hall, R. J.; Altizer, S. On the relationship between body condition and parasite infection in wildlife: a review and meta-analysis. *Ecol. Lett.* **2018**, (doi:10.1111/ele.13160)
- Santoro, M.; Mattiucci, S.; Work, T.; Cimmaruta, R.; Nardi, V.; Cipriani, P.; Bellisario, B.; Nascetti, G. Parasitic infection by larval helminths in Antarctic fishes: pathological changes and impact on the host body condition index. *Dis. Aquat. Organ.* **2013**, *105*, 139–148. (doi:10.3354/dao02626)
- Sanz, A.; Hidalgo, M. C.; Morales, A. E.; Cillero, C.; Domenzain, A.; Domenzain, J.; García-Gallego, M. Evolution of antioxidant defenses and lipid peroxidation with age in the sturgeon *Acipenser naccarii*. *Proceedings of the 4th International Symposium on Sturgeon* **2001**, July 8–13. Oshkosh, Wisconsin, USA, p. 89.

- Saurabh, S.; Sahoo, P. K. Lysozyme: an important defence molecule of fish innate immune system. *Aquac. Res.* **2008**, *39*, 223–239. (doi:10.1111/j.1365-2109.2007.01883.x)
- Sebastiano, M.; Angelier, F.; Blevin, P.; Ribout, C.; Sagerup, K.; Descamps, S.; Herzke, D.; Moe, B.; Barbraud, C.; Bustnes, J. O.; Gabrielsen, G. W.; Chastel, O. Exposure to PFAS is associated with telomere length dynamics and demographic responses of an arctic top predator. *Environ. Sci. Technol.* **2020**, *54*, 10217–10226. (doi:10.1021/acs.est.0c03099)
- Seine-Normandie Water Agency. Water Management in the Seine-Normandie River basin district, **2011**. http://www.eau-seine-normandie.fr/sites/public_file/docutheque/2017-03/4VoletsAESN_ENG.pdf
- Shekhova, E. Mitochondrial reactive oxygen species as major effectors of antimicrobial immunity. *PLoS Pathog.* **2020**, *16*, e1008470. (doi:10.1371/journal.ppat.1008470)
- Silva-Gomes, A. L.; Coelho-Filho, J. G.; Viana-Silva, W.; Braga-Oliveira, M. I.; Bernardino, G.; Costa, J. I. The impact of *Neoechinorhynchus buttnerae* (Golvan, 1956) (Eoacanthocephala: Neochinorhynchidae) outbreaks on productive and economic performance of the tambaqui *Colossoma macropomum* (Cuvier, 1818), reared in ponds. *Lat. Am. J. Aquat. Res.* **2017**, *45*, 496–500. (doi:10.3856/vol45-issue2-fulltext-25)
- Singh, A.; Kukreti, R.; Saso, L.; Kukreti, S. Oxidative stress: role and response of short guanine tracts at genomic locations. *Int. J. Mol. Sci.* **2019**, *20*, 4258. (doi:10.3390/ijms20174258)
- Siddall, R.; Sures, B. Uptake of lead by *Pomphorhynchus laevis* cystacanths in *Gammarus pulex* and immature worms in chub (*Leuciscus cephalus*). *Parasitol. Res.* **1998**, *84*, 573–577. (doi:10.1007/s004360050451)
- Simide, R.; Angelier, F.; Gaillard, S.; Stier, A. Age and heat stress as determinants of telomere length in a long-lived fish, the Siberian sturgeon. *Physiol. Biochem. Zool.* **2016**, *89*, 441–447. (doi:10.1086/687378)
- Skouras, A.; Broeg, K.; Dizer, H.; von Westernhagen, H.; Hansen, P. D.; Steinhagen, D. The use of innate immune responses as biomarkers in a programme of integrated biological effects monitoring on flounder (*Platichthys flesus*) from the southern North Sea. *Helgoland Mar Res* **2003**, *57*, 190–198. (doi:10.1007/s10152-003-0141-7)
- Sletten, S.; Bourgeon, S.; Bårdsen, B. J.; Herzke, D.; Criscuolo, F.; Massemin, S.; Zahn, S.; Johnsen, T. V.; Bustnes, J. O. Organohalogenated contaminants in whitetailed eagle (*Haliaeetus albicilla*) nestlings: an assessment of relationships to immunoglobulin levels, telomeres and oxidative stress. *Sci. Total Environ.* **2016**, *539*, 337e349. (doi:10.1016/j.scitotenv.2015.08.123)
- Snyder, S. M.; Pulster, E. L.; Murawski, S. A. Associations between chronic exposure to polycyclic aromatic hydrocarbons and health indices in Gulf of Mexico Tilefish (*Lopholatilus chamaeleonticeps*) post- Deepwater Horizon. *Environ. Toxicol. Chem.* **2019**, *38*, 2659–2671. (doi:10.1002/etc.4583)
- Soler-Jiménez, L. C.; Hernández-Núñez, E.; Velázquez-Abunader, I.; Centeno-Chalé, A.; Vidal-Martínez, V. M. Polycyclic aromatic hydrocarbons in the cestodes *Oncomegas wagneri* parasite of Mexican flounder *Cyclopsetta chittendeni*. *Parasitol Res.* **2020**, *119*, 903–913. (doi:10.1007/s00436-019-06597-z)
- Soltani, T.; Safahieh, A.; Zolgharnien, H.; Matroodi, S. Interactions of oxidative DNA damage and CYP1A gene expression with the liver enzymes in Klunzinger's mullet exposed to benzo[a]pyrene. *Toxicol. Reports* **2019**, *6*, 1097–1103. (doi:10.1016/j.toxrep.2019.10.013)
- Sorci, G.; Faivre, B. Inflammation and oxidative stress in vertebrate host-parasite systems. *Philos. T. R. Soc. B* **2009**, *364*, 71–83. (doi:10.1098/rstb.2008.0151)
- Spann, N.; Goedkoop, W.; Traunspurger, W. Phenanthrene bioaccumulation in the nematode *Caenorhabditis elegans*. *Environ. Sci. Technol.* **2015**, *49*, 1842–1850.

- Staples, C. A.; Peterson, D. R.; Parkerton, T. F.; Adams, W. J. The environmental fate of phthalate esters: A literature review. *Chemosphere* **1997**, *35*, 667–749. (doi:10.1016/s0045-6535(97)00195-1)
- Stauffer, J.; Panda, B.; Eeva, T.; Rainio, M.; Ilmonen, P. Telomere damage and redox status alterations in free-living passerines exposed to metals. *Sci. Total Environ.* **2017**, *575*, 841e848. (doi:10.1016/j.scitotenv.2016.09.131)
- Stegcman, J. J.; Pajor, A. M.; Thomas, P. Influence of estradiol and testosterone on cytochrome P-450 in fish and monooxygenase activity in immature brook trout, *Salvelinus fontinalis*. *Biochem. Pharmacol.* **1982**, *31*, 3979–3989.
- Stoscheck, C. M. Quantitation of protein. *Methods Enzymol.* **1990**, *182*, 50–68.
- Stow, C. A.; Jackson, L. J.; Amrhein, J. F. An examination of the PCB:lipid relationship among fish individuals. *Can. J. Fish. Aquat. Sci.* **1997**, *54*: 1031–1038.
- Strona, G. Past, present and future of host–parasite co-extinctions. *Int. J. Parasitol.* **2015**, *4*, 431–441. (doi:10.1016/j.ijppaw.2015.08.007)
- Struch, R. E.; Pulster, E. L.; Schreier, A. M.; Murawski, S. A. Hepatobiliary analyses suggest chronic PAH exposure in Hakes (*Urophycis* spp.) following the deepwater horizon oil spill. *Environ. Toxicol. Chem.* **2019**. (doi:10.1002/etc.4596)
- Studnicka, M.; Siwicki, A.; Ryka, B. Lysozyme level in carp (*Cyprinus carpio* L.). *Bamidgeh* **1986**, *38*, 22–25.
- Sudyka, J.; Podmokła, E.; Drobniak, S. M.; Dubiec, A.; Arct, A.; Gustafsson, L.; Cichoń, M. Sex-specific effects of parasites on telomere dynamics in a short-lived passerine—the blue tit. *The Science of Nature* **2019**, *106*. (doi:10.1007/s00114-019-1601-5)
- Sueiro, M. C.; Bagnato, E.; Palacios, M. G. Parasite infection and immune and health-state in wild fish exposed to marine pollution. *Mar. Pollut. Bull.* **2017**, *119*, 320–324. (doi:10.1016/j.marpolbul.2017.04.011)
- Sures, B.; Taraschewski, H.; Jackwerth, E. Lead accumulation in *Pomphorhynchus laevis* and its host. *J. Parasitol.* **1994**, *80*, 355. (doi:10.2307/3283403)
- Sures, B.; Taraschewski, H. Cadmium concentrations in two adult acanthocephalans, *Pomphorhynchus laevis* and *Acanthocephalus lucii* as compared with their fish hosts and cadmium and lead levels in larvae of *A. lucii* as compared with their crustacean host. *Parasitol Res.* **1995**, *81*, 494–497. (doi:10.1007/BF00931792)
- Sures, B.; Siddall, R. *Pomphorhynchus laevis*: the intestinal acanthocephalan as a lead sink for its fish host, chub (*Leuciscus cephalus*). *Exp. Parasitol.* **1999**, *93*, 66–72. (doi:10.1006/expr.1999.4437)
- Sures, B.; Siddall, R.; Taraschewski, H. Parasites as accumulation indicators of heavy metal pollution. *Parasitol. Today* **1999**, *15*, 16–21. (doi:10.1016/S0169-4758(98)01358-1)
- Sures, B.; Jürges, G.; Taraschewski, H. Accumulation and distribution of lead in the archiacanthocephalan *Moniliformis moniliformis* from experimentally infected rats. *Parasitology* **2000**, *121*, 427–433. (doi:10.1017/s003118209900654x)
- Sures, B. Competition for minerals between *Acanthocephalus lucii* and its definitive host perch (*Perca fluviatilis*). *Int. J. Parasitol.* **2002**, *32*, 1117–1122. (doi:10.1016/s0020-7519(02)00083-8)
- Sures, B.; Dezfuli, B. S.; Krug, H. F. The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) interferes with the uptake and accumulation of lead (210Pb) in its fish host chub (*Leuciscus cephalus*). *Int. J. Parasitol.* **2003**, *33*, 1617–1622. (doi:10.1016/S0020-7519(03)00251-0)
- Sures, B.; Siddall, R. *Pomphorhynchus laevis* (Palaeacanthocephala) in the intestine of chub (*Leuciscus cephalus*) as an indicator of metal pollution. *Int. J. Parasitol.* **2003**, *33*, 65–70. (doi:10.1016/s0020-7519(02)00249-7)

- Sures, B. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends Parasitol.* **2004**, 20, 170–177. (doi:10.1016/j.pt.2004.01.014)
- Sures, B.; Radszuweit, H. Pollution-induced heat shock protein expression in the amphipod *Gammarus roeseliis* affected by larvae of *Polymorphus minutus* (Acanthocephala). *J. Helmintho.* **2007**, 181, 191–197. (doi:10.1017/S0022149X07751465)
- Sures, B.; Nachev, M.; Selbach, C.; Marcogliese, D. J. Parasite responses to pollution: what we know and where we go in “Environmental Parasitology.” *Parasite Vector* **2017**, 10, 65. (doi:10.1186/s13071-017-2001-3)
- Suthers, I. Significance of larval condition: comment on laboratory experiments. *Can. J. Fish. Aquat. Sci.* **2000**, 57, 1534–1536. (doi:10.1139/cjfas-57-7-1534)
- Syväranta, J.; Lensu, A.; Marjomäki, T. J.; Oksanen, S.; Jones, R. I. An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. *PLoS ONE* **2013**, 8, e56094. (doi:10.1371/journal.pone.0056094)
- Taraschewski, H. Host-parasite interactions in Acanthocephala: a morphological approach. *Adv. Parasitol* **2000**, 46, 1–179. (doi:10.1016/s0065-308x(00)46008-2)
- Teil, M. J.; Tlili, K.; Blanchard, M.; Chevreuil, M.; Alliot, F.; Labadie, P. Occurrence of polybrominated diphenyl ethers, polychlorinated biphenyls, and phthalates in freshwater fish from the Orge River (Ile-de France). *Arch. Environ. Contam. Toxicol.* **2012**, 63, 101–113. (doi:10.1007/s00244-011-9746-z)
- Tenji, D.; Micic, B.; Sipos, S.; Miljanovic, B.; Teodorovic, I.; Kaisarevic, S. Fish biomarkers from a different perspective: evidence of adaptive strategy of *Abramis brama* (L.) to chemical stress. *Environ. Sci. Eur.* **2020**, 32, 47. (doi:10.1186/s12302-020-00316-7)
- Thielen, F.; Zimmermann, S.; Baska, F.; Taraschewski, H.; Sures, B. The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) from barbel as a bioindicator for metal pollution in the Danube River near Budapest, Hungary. *Environ. Pollut.* **2004**, 129, 421–429. (doi: 10.1016/j.envpol.2003.11.011)
- Thilakarathne, I. D. S. I. P.; McLaughlin, J. D.; Marcogliese, D. J. Effects of pollution and parasites on biomarkers of fish health in spottail shiners *Notropis hudsonius* (Clinton). *J. Fish Biol.* **2007**, 71, 519–538. (doi:10.1111/j.1095-8649.2007.01511.x)
- Timi, J. T.; Poulin, R. Why ignoring parasites in fish ecology is a mistake. *Int. J. Parasitol.* **2020**. (doi:10.1016/j.ijpara.2020.04.007)
- Torres, J.; Eira, C.; Miquel, J.; Foronda, P.; Feliu, C. Cadmium and lead concentrations in *Moniliformis moniliformis* (Acanthocephala) and *Rodentolepis microstoma* (Cestoda), and in their definitive hosts, *Rattus rattus* and *Mus domesticus* in El Hierro (Canary Archipelago, Spain). *Acta Parasitol.* **2011**, 56, 320–4. (doi:10.2478/s11686-011-0064-4)
- Trenzado, C.; Hidalgo, M. C.; García-Gallego, M.; Morales, A. E.; Furné, M.; Domezain, A.; Domezain, J.; Sanz, A. Antioxidant enzymes and lipid peroxidation in sturgeon *Acipenser naccarii* and trout *Oncorhynchus mykiss*. A comparative study. *Aquaculture* **2006**, 254, 758–767. (doi:10.1016/j.aquaculture.2005.11.020)
- Trubiroha, A.; Kroupova, H.; Frank, S. N.; Sures, B.; Kloas, W. Inhibition of gametogenesis by the cestode *Ligula intestinalis* in roach (*Rutilus rutilus*) is attenuated under laboratory conditions. *Parasitology* **2010**, 138, 648–659. (doi:10.1017/s0031182010001514)
- Tuvikene, A. Responses of fish to polycyclic aromatic hydrocarbons (PAHs). *Ann. Zool. Fennici.* **1995**, 32, 295–309.
- Ünver, B.; Erk’akan, F. Diet composition of chub, *Squalius cephalus* (Teleostei: Cyprinidae), in Lake Tödürge, Sivas, Turkey. *J. Appl. Ichthyol.* **2011**, 27, 1350–1355. (doi:10.1111/j.1439-0426.2011.01766.x)

- US EPA (United States Environmental Protection Agency). Estimation Programs Interface Suite (EPI Suite) for Microsoft Windows, v 4.10. Washington, DC, USA, **2012**; <http://www.epa.gov/opptintr/exposure/pubs/episuitedl.htm>.
- Uribe, C.; Folch, H.; Enriquez, R.; Moran, G. Innate and adaptive immunity in teleost fish: a review. *Veterinarni Medicina* **2011**, 56, 486–503. (doi:10.17221/3294-VETMED)
- Valton, A. S.; Serre-Dargnat, C.; Blanchard, M.; Alliot, F.; Chevreuil, M.; Teil, M. J. Determination of phthalates and their by-products in tissues of roach (*Rutilus rutilus*) from the Orge river (France). *Environ. Sci. Pollut. R.* **2014**, 21, 12723–12730. (doi:10.1007/s11356-014-3213-0)
- Varanasi, U.; Stein, J. E.; Nishimoto, M. *Biotransformation and deposition of polycyclic aromatic hydrocarbons (PAH) in fish*. In: Varanasi, U. (Ed.), *Metabolism of Polycyclic Aromatic Hydrocarbon in the Aquatic Environment*. CRC Press Inc., Boca Raton, **1989**, pp. 93–150.
- Vidal-Martínez, V. M.; Aguirre-Macedo, M. L.; Noreña-Barroso, E.; Gold-Bouchot, G.; Caballero-Pinzón, P. I. Potential interactions between metazoan parasites of the Mayan catfish *Ariopsis assimilis* and chemical pollution in Chetumal Bay, Mexico. *J. Helminthol.* **2003**, 77, 173–184. (doi: 10.1079/JOH2002158)
- Vidal-Martínez, V. M.; Pech, D.; Sures, B.; Purucker, S. T.; Poulin, R. Can parasites really reveal environmental impact? *Trends Parasitol.* **2009**, 26, 44–51. (doi:10.1016/j.pt.2009.11.001)
- Vives, I.; Grimalt, J. O.; Ventura, M.; Catalan, J.; Rosseland, B. O. Age dependence of the accumulation of organochlorine pollutants in brown trout (*Salmo trutta*) from a remote high mountain lake (Redó, Pyrenees). *Environ. Poll.* **2005**, 133, 343–350. (doi:10.1016/j.envpol.2004.05.027)
- Vuorinen, P. J.; Keinänen, M.; Vuontisjärvi, H.; Baršienė, J.; Broeg, K.; Förlin, L.; ... Schiedek, D. Use of biliary PAH metabolites as a biomarker of pollution in fish from the Baltic Sea. *Mar. Pollut. Bull.* **2006**, 53, 479–487. (doi:10.1016/j.marpolbul.2005.11.020)
- Walk, S. T.; Blum, A. M.; Ewing, S. A.-S.; Weinstock, J. V.; Young, V. B. Alteration of the murine gut microbiota during infection with the parasitic helminth *Heligmosomoides polygyrus*. *Inflamm. Bowel Dis.* **2010**, 16, 1841–1849. (doi:10.1002/ibd.21299)
- Wang, L. R.; Wang, Y.; Chen, J. W.; Guo, L. H. A structurebased investigation on the binding interaction of hydroxylated polycyclic aromatic hydrocarbons with DNA. *Toxicology* **2009**, 262, 250–257.
- Weinersmith, K. L.; Earley, R. L. Better with your parasites? Lessons for behavioural ecology from evolved dependence and conditionally helpful parasites. *Anim. Behav.* **2016**, 118, 123–133. (doi:10.1016/j.anbehav.2016.06.004)
- Whittemore, K.; Vera, E.; Martínez-Nevado, E.; Sanpera, C.; Blasco, M. A. Telomere shortening rate predicts species life span. *P. Natl. Acad. Sci. USA* **2019**, 116, 15122–15127. (doi:10.1073/pnas.1902452116)
- Whyte, J. J.; Jung, R. E.; Schmitt, C. J.; Tillitt, D. E. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit. Rev. Toxicol.* **2000**, 30, 347–570. (doi:10.1080/10408440091159239)
- Wilhelm Filho, D.; Torres, M. A.; Tribess, T. B.; Pedrosa, R. C.; Soares, C. H. L. Influence of season and pollution on the antioxidant defenses of the cichlid fish acará (*Geophagus brasiliensis*). *Braz. J. Med. Biol. Res.* **2001**, 34, 719–726. (doi:10.1590/s0100-879x2001000600004)
- Williams, G. M.; Iatropoulos, M. J. Alteration of liver cell function and proliferation: differentiation between adaptation and toxicity. *Toxicol. Pathol.* **2002**, 30, 41–53.
- Winston, G. W.; Di Giulio, R. T. Prooxidant and antioxidant mechanisms in aquatic organisms. *Aquat. Toxicol.* **1991**, 19, 137–161. (doi:10.1016/0166-445x(91)90033-6)

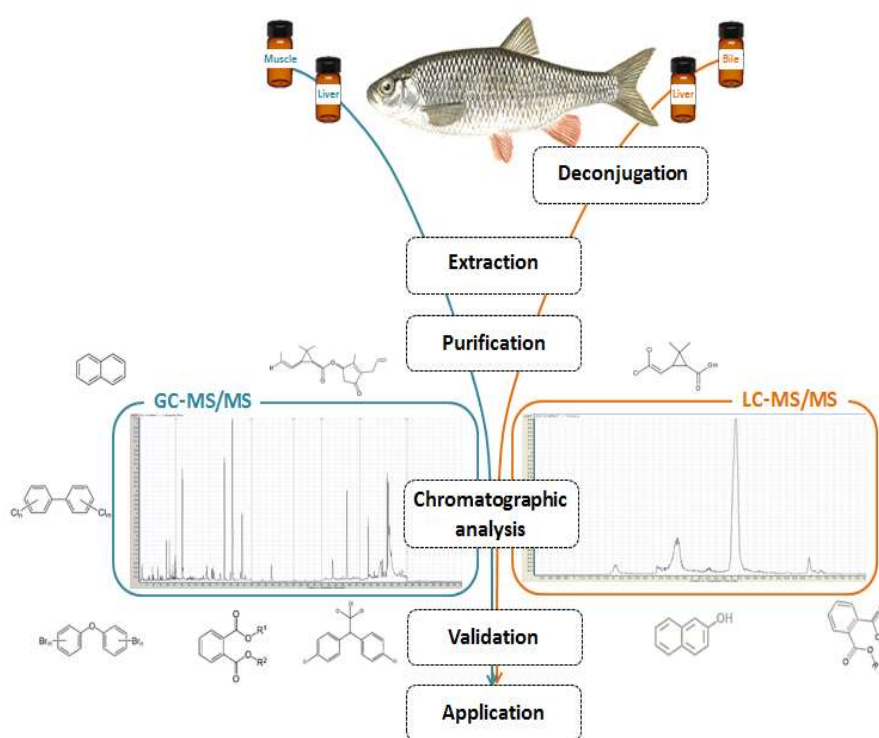
- Wu, J. C.; Lai, C. S.; Tsai, M. L.; Ho, C. T.; Wang, Y. J.; Pan, M. H. Chemopreventive effect of natural dietary compounds on xenobiotic-induced toxicity. *J. Food Drug Anal.* **2017**, *25*, 176–186. (doi:10.1016/j.jfda.2016.10.019)
- WWF. *Living Planet Report - 2018: Aiming Higher*. Grooten, M.; Almond, R. E. A.(Eds). WWF, Gland, Switzerland, **2018**.
- Xin, X.; Senthilkumar, P. K.; Schnoor, J. L.; Ludewig, G. Effects of PCB126 and PCB153 on telomerase activity and telomere length in undifferentiated and differentiated HL-60 cells. *Environ. Sci. Pollut. R.* **2015**, *23*, 2173–2185. (doi:10.1007/s11356-015-5187-y)
- Yu, M.; Luo, X. J.; Wu, J. P.; Chen, S. J.; Mai, B. X. Bioaccumulation and trophic transfer of polybrominated diphenyl ethers (PBDEs) in biota from the Pearl River Estuary, South China. *Environ. Int.* **2009**, *35*, 1090-109. (doi:10.1016/j.envint.2009.06.007)
- Yue, N.; Deng, C.; Li, C.; Wang, Q.; Li, M.; Wang, J.; Jin, F. The occurrence and distribution of phthalate esters and their major metabolites in porcine tissues. *J. Agr. Food Chem.* **2020**. (doi:10.1021/acs.jafc.9b07643)
- Zhao, Y.; Liub, H.; Wangb, Q.; Lia, B.; Zhangb, H.; Pi, Y. The effects of benzo[a]pyrene on the composition of gut microbiota and the gut health of the juvenile sea cucumber *Apostichopus japonicus* Selenka. *Fish Shellfish Immun.* **2019**, *93*, 369–379. (doi:10.1016/j.fsi.2019.07.07)

VIII – Appendix

1. Annex 1

Article 1

Molbert N, Alliot F, Santos R, Chevreuil M, Mouchel JM, Goutte A. 2019 Multi-residue methods for the determination of organic micropollutants and their metabolites in fish tissues. *Environ. Toxicol. Chem.* **38**, 1866–1878. (doi:10.1002/etc.4500)



Multiresidue Methods for the Determination of Organic Micropollutants and Their Metabolites in Fish Matrices

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Abstract: Two analytical methods were developed for the determination of 48 organic compounds and 20 of their main by-products in fish matrices. The targeted compounds belong to various chemical classes of metabolizable (phthalates, polycyclic aromatic hydrocarbons, insecticides [pyrethroids and *N,N*-diethyl-meta-toluamide]) and legacy (organochlorine pesticides, polychlorinated biphenyls, polybrominated diphenyl ethers) pollutants. Analyses were performed by gas and liquid chromatography–tandem mass spectrometry in multiple reaction monitoring (MRM) and dynamic MRM, respectively. Method performances were satisfactory, with results meeting the validation criteria because they achieved good linearity responses, recovery, precision, and accuracy for most of the 68 investigated compounds. The methods were then applied on 3 feral chub (*Squalius cephalus*) collected from the Marne hydrographic network (France). Twenty-six parent compounds and 5 metabolites were systematically detected in fish matrices, with substantial concentration variability within and among individuals. Phthalates and pyrethroids accounted for most of the pollutant load. Metabolite concentrations in liver samples exceeded those of parent molecules in fish muscle. The present study presents 2 reliable methods for the determination of a wide range of contaminants and underlines the importance of metabolite analysis for a more comprehensive understanding of pollutant bioaccumulation and fate in aquatic organisms. *Environ Toxicol Chem* 2019;00:1–13. © 2019 SETAC

Keywords: Persistent organic micropollutants; Phthalates; By-products; Polycyclic aromatic hydrocarbons; Pyrethroid; Flame retardants

INTRODUCTION

Environmental release of organic pollutants through agricultural, industrial, and urban sources may erode freshwater biodiversity and jeopardize ecosystem functioning (Vörösmarty et al. 2010; Hamilton et al. 2016). Fish species are often used to assess environmental contamination because their feeding strategies, life-history traits (long life span), and interactions with the surrounding environment (e.g., water and sediments) provide an integrated view of the aquatic pollution (Van der Oost et al. 2003). In Europe, polycyclic aromatic hydrocarbons (PAHs), pesticides, and plasticizers raise important concerns to freshwater ecosystems (Malaj et al. 2014). Even though fish have the capacity to metabolize and excrete those pollutants (Barron et al. 1995), their biotransformation

can generate toxic metabolites, which have been largely neglected in freshwater monitoring (Fourgous et al. 2016). In addition, legacy chemicals (organochlorine pesticides [OCPs], polychlorinated biphenyls [PCBs], and polybrominated diphenyl ethers [PBDEs]) are still being studied (Ondarza et al. 2014; Couderc et al. 2015) because their persistence and toxicity could represent a risk to the environment and human health (Carpenter 2011).

Because pollutants are mainly found in mixtures, there is a need to routinely measure as many organic compounds as possible, including their metabolites. Most of the existing analytical methods applied to aquatic organisms are restricted to a few groups of chemicals (Teil et al. 2012; Chatterje et al. 2016; Nagyová and Tölgyessy 2019), and none of them include both PAHs and plasticizers, which are primarily responsible for the poor chemical status of surface water bodies in Europe (European Environment Agency 2019). In 2013, Environmental Quality Standards for biota (EQS_{biota}) were defined for 11 hazardous pollutants (European Commission 2014) to protect

This article contains online-only Supplemental Data.

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Published online 20 May 2019 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/etc.4500

freshwater ecosystems, as well as human health, from the potential adverse effects of chemicals. However, current methods are not sufficient for compliance monitoring because of unreliable limits of quantification (Malaj et al. 2014). A challenging task is to develop analytical methods presenting high sensitivity and low limits of quantification to detect and quantify a larger set of hazardous compounds at trace concentrations in complex biological matrices.

The present study aimed to develop 2 analytical methods to quantify 1) 48 compounds, belonging to 6 families of organic pollutants (phthalates, PAHs, PCBs, PBDEs, OCPs, and insecticides [pyrethroids and *N,N*-diethyl-meta-toluamide {DEET}]) and 2) 20 of their metabolites in fish matrices using gas and liquid chromatography coupled to tandem mass spectrometry. A total of 68 compounds were targeted, including 21 listed as priority and hazardous substances (European Commission 2014). Method performances were evaluated on muscle and liver tissues for parent compounds and on bile and liver samples for metabolites because of their nature (fluid vs tissue) and biological role (biotransformation and excretion). Once validated, these methods were applied on various fish matrices (i.e., gill, muscle, liver, stomach content, gonad, and bile) of the European chub, *Squalius cephalus*, sampled from the Marne hydrographic network (France), known to be under agricultural and urban pressures.

MATERIALS AND METHODS

Materials and reagents

Specific equipment (glassware and polycarbonate tubes) was used for sample storage to minimize phthalates contamination. Glassware was washed twice with acetone: *n*-hexane (1:1, v/v) and then heated for 4 h at 400 °C. The equipment was stored with glass stoppers and wrapped in aluminum foil. Automated analytical glass syringes (ThermoFisher Scientific) were used for microvolumes. Oasis hydrophilic–lipophilic balance (HLB; 6 mL/200 mg) and Florisil (Supelco; 1 g/6 mL) cartridges were provided by Waters and Sigma-Aldrich, respectively. Acetone, *n*-hexane, dichloromethane, and ethyl acetate were supplied by Merck. Ammonium acetate, phosphoric acid, and isopropanol were purchased from Sigma-Aldrich. Acetonitrile and methanol were purchased from VWR Chemicals. Formic acid was provided by Carlo Erba Reagents, and ultrapure water (mqH₂O) was from a Milli-Q system. 4-Methylumbelliferone and 4-methylumbelliferol-β-D-glucuronide were obtained from LGC Standards. β-Glucuronidase (*Escherichia coli* K12) was provided by Sigma-Aldrich. Helium and nitrogen (99.999%) were supplied by Air Liquide. All solvents were of gas and liquid chromatography quality and free from phthalate residues.

Target analytes

In the present study, 16 PAHs (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), 7 phthalate esters (dimethyl phthalate [DMP], diethyl phthalate [DEP], *n*-butyl benzyl phthalate [BBP], di-*n*-butyl phthalate [DnBP], di-*iso*-butyl phthalate [DiBP], di-2-ethylhexyl phthalate [DEHP], and di-*n*-octyl phthalate [DnOP]), 7 PCBs (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, and PCB-180), 6 PBDEs (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154), 4 OCPs (pentachlorobenzene PeCB), hexachlorobenzene [HCB], lindane [γ-HCB], and dichlorodiphenyldichloroethylene [*p,p'*-DDE]), 7 pyrethroids (bifenthrin, permethrin, phenothrin, cyfluthrin, cypermethrin, fenvalerate, and deltamethrin), and DEET were analyzed. For metabolites, 7 phthalate monoesters (mono-methyl phthalate [MMP], mono-ethyl phthalate [MEP], mono-*iso*-butyl phthalate [MiBP], mono-*n*-butyl phthalate [MnBP], mono-benzyl phthalate [MBzP], mono-*n*-octyl phthalate [MnOP], and mono-2-ethylhexyl phthalate [MEHP]), 2 oxidized by-products (mono-2-ethyl-5-oxohexyl phthalate and mono-2-ethyl-5-hydroxyhexyl phthalate [MEHHP]), 11 hydroxylated metabolites of polycyclic aromatic hydrocarbon (OH-PAH; 1-hydroxynaphthalene, 2-hydroxynaphthalene [2-OH-Nap], 2-hydroxyfluorene [2-OH-Flu], 3-OH-Flu, 9-OH-Flu, 1-hydroxyphenanthrene [1-OH-Phe], 2-OH-Phe, 3-OH-Phe, 4-OH-Phe, 1-hydroxypyrene [1-OH-Pyr], 6-hydroxychrysene [6-OH-Chr], and 3-hydroxybenzo[*a*]pyrene [3-OH-BaP]), and 4 pyrethroid metabolites (3-[2,2-dichlorovinyl]-2,2-dimethyl-1-cyclopropane [*cis*-DCCA and *trans*-DCCA], 4-fluoro-3-phenoxybenzoic acid [4-FPBA], and 3-phenoxybenzoic acid [3-PBA]) were targeted.

Standards

A mixed standard solution of PAHs dissolved in cyclohexane at 10 ng/μL each was purchased from Cluzeau Info Labo. A standard solution of phthalate esters in iso-octane at 1000 ng/μL, OCPs at 100 ng/μL each, pyrethroids in iso-octane at different concentrations (10–200 ng/μL), PCBs in iso-octane at 10 ng/μL, and PBDEs in nonane at 1 ng/μL was provided by LGC Standards. Isotope-labeled compounds, used as internal standards, were purchased from Cluzeau Info Labo for phenanthrene-*d*₁₀, pyrene-*d*₁₀, benzo[*a*]anthracene-*d*₁₂, PCB-30, and PCB-107, from LGC Standards for DEP-*d*₄, DEHP-*d*₄, ¹³C-permethrin, and lindane-*d*₆ and from BCP Instruments for ¹³C₁₂-BDE-47 and ¹³C₁₂-BDE-153. All mixtures of internal standards for metabolite analysis were purchased from LGC Standards: MnBP-¹³C₁₂, MEHP-¹³C₁₂, and mono-*iso*-nonyl phthalate-¹³C₁₂, each one at 25 ng/μL in acetonitrile, *trans*-DCCA-*d*₃, ¹³C₆-3-PBA, and ¹³C₆-4-FPBA at 100 μg/mL in acetonitrile, and 5 OH-PAHs (2-OH-Nap-¹³C₆, 1-OH-Pyr-¹³C₆, 9-OH-Flu-¹³C₆, 1-OH-Phe-¹³C₆, and 6-OH-Chr-¹³C₆), each one at 50 μg/mL in toluene. For each contaminant family (parent compounds and metabolites), the quantity of internal standards spiked per sample is listed in Supplemental Data, Table S1.

Sample collection

The European chub, *S. cephalus*, is one of the most abundant freshwater fish species in France and is widely distributed through most of northern Eurasia. Its distribution and

TABLE 1: Morphological and biological data of chub *Squalius cephalus* sampled in the Marne hydrographic network, France

	Chub 1	Chub 2	Chub 3
Length (cm)	33.9	41.5	40.5
Weight (g)	442	760	862
Date	7/9/16	16/9/16	19/9/16
Sampling location	Verneuil	Celles-les-condé	Tresmes
Coordinates	49°5'42''N, 3°40'23''E	49°0'32''N, 3°34'8''E	48°48'46''N, 2°59'30''E
Lipid content (%)			
Gill	15.4	6.65	6.32
Liver	14.5	3.19	5.89
Stomach content	3.35	19.8	11.7
Muscle	6.49	3.09	6.51
Gonad	5.66	2.18	3.01

abundance, large size, wide ecological niche, as well as tolerance to pollution represent well-suited characteristics to assess anthropogenic pollution (Hájková et al. 2007). Chubs were collected in 3 tributaries of the Marne hydrographic network, France (Table 1) located in agricultural and densely populated urban areas. Fish were caught by electrofishing in September 2016, anesthetized with tricaine methane sulfonate (1 g/L), and euthanized. Only the largest individuals ($n = 3$ females; Table 1) from each sampling site were used to obtain enough biological material (muscle, gill, gonad, stomach content, liver, and bile) for the quantification of parent compounds or metabolites. Biological material was stored at -20°C until further treatment.

Ethics statement

The authorization required by Article L436-9 of the Environment Code for the exceptional capture of fish for scientific purposes was provided by local administration authorities (Departmental Direction of Territories). More precisely, electrofishing was carried out in full accordance with the French Environment Policy (including water policy) and European standards (EN14011).

Sample extraction and purification

Analyses of parent compounds were adapted from Sánchez-Avila et al. (2011), whereas for metabolites, the procedure was adapted from Frederiksen et al. (2010) and Zhang et al. (2015).

Parent compounds. Before extraction, samples were lyophilized (Alpha 1-4 LDplus; Christ) for 48 h and then ground to obtain a fine powder. Freeze-dried muscle (0.5 g), liver (0.1 g), gill (0.5 g), gonad (0.5 g), and stomach content (0.5 g) samples were spiked with a mixture of all internal standards (Supplemental Data, Table S1) and stored overnight at 4°C . Solid-liquid extractions were performed by accelerated solvent extraction (ASE 350; Thermo Fisher Scientific) programmed to operate 3 extractions (5 min, 100°C) and purging cycles (2 min, solvent flush: 100% of cell volume) using different solvents: hexane:dichloromethane (1:1, v/v) and hexane:acetone (1:1, v/v). The extracts were concentrated to approximately 1 mL using the Genevac™

Concentrator EZ-2 (Biopharma Technologies) and then down to 0.5 mL under a nitrogen stream at 62°C . The extracts were then purified using Florisil cartridges (Supelco; 1 g/6 mL) previously conditioned with 2×5 mL of hexane:acetone (1:1, v/v) and 2×5 mL of hexane:dichloromethane (1:1, v/v). The sample extract was eluted with 2×5 mL of hexane:dichloromethane (1:1, v/v) and 2×5 mL of hexane:acetone (1:1, v/v). Prior to analysis, the extracts were concentrated using EZ-2, and the remaining solvents were evaporated under a nitrogen stream (62°C). For each sample, a freeze-dried aliquot of 0.1 g of muscle, stomach content, liver, gonad, and gill was analyzed to determine its lipid content. Briefly, fish matrices were sonicated for 15 min with 2×5 mL of hexane:isopropanol (60:40, v/v) and then centrifuged (10 min, 2500 rpm; Labadie et al. 2010). The supernatant was deposited on a Vivaclear centrifugal filter (0.8 μm ; Sartorius) and rinsed twice with 0.2 mL of hexane:isopropanol (60:40, v/v). Extracts were evaporated under a nitrogen stream (62°C), and the residue was weighed to the nearest 0.1 mg.

Metabolites in solid matrix. Ammonium acetate (2 mL, 1 M, pH 6.5) and 4-methylumbelliferyl- β -D-glucuronide (10 μL at 57.5 ng/ μL ; evaluates the deconjugation reaction) were added to freeze-dried liver (0.1 g) samples. The mixture was then vortexed (for 15 s), followed by 10 min of ultrasound. Once 10 μL of β -glucuronidase was added (from *E. coli* K12) to the mixture, a SW22 heated shaker water bath (90 min, 37°C , 170 rpm; Julabo) was used for the deconjugation step. The deconjugated sample was spiked with a mixture of all surrogates and 10 μL of a 100 ng/ μL 4-methylumbelliferone- $^{13}\text{C}_{12}$. The addition of 1 mL mqH_2O 1% phosphoric acid terminated the reaction. After homogenization and centrifugation (4500 rpm, 5 min), the supernatant was collected and extracted on solid-phase extraction cartridges (Oasis HLB 6 mL/200 mg) previously conditioned (5 mL acetonitrile, 5 mL mqH_2O , 5 mL ammonium acetate 0.15 M, pH 2–3). After loading, the samples were rinsed with 5 mL ammonium acetate (0.15 M) and washed with 5 mL of 1% phosphoric acid in mqH_2O + 5 mL mqH_2O : acetonitrile (90:10, v/v). After drying for 10 min, the samples were eluted with 2×5 mL of acetonitrile and 2×5 mL of ethyl acetate. The extracts were concentrated using EZ-2 (35 min), evaporated until almost dryness under a nitrogen stream (62°C), and re-suspended with 200 μL of 0.1% formic acid in methanol. The re-suspended solution was filtered (0.2 μm , nylon; VWR Chemicals) under centrifugation (5000 rpm, 1 min), rinsed, and transferred in an amber vial. All extracts were stored at -20°C until use.

Metabolites in liquid matrix. Ammonium acetate (2 mL, 1 M, pH 6.5) and 10 μL of a 57.5 ng/ μL 4-methylumbelliferyl- β -D-glucuronide were added to bile (50–100 μL) samples. The mixture was then vortexed (for 15 s), followed by 10 min of ultrasound. The deconjugation and extraction steps were as described in section, *Metabolites in solid matrix*.

Instruments and analytical conditions

Gas chromatography–tandem mass spectrometry. Analyses of parent compounds were performed with an Agilent 7890 A gas chromatograph (GC) coupled to a 7000 B triple-quadrupole mass

spectrometer (MS/MS; Agilent Technologies). Electron ionization (+70 eV) was used to characterize the targeted compounds in the gas phase with the ionization source set at 250 °C. Molecules were separated on a Zebron SemiVolatile analytical column (30 m, 0.25 mm inner diameter × 0.25 μm film thickness; Phenomenex) connected to a Restek deactivated silica guard column (0.25 mm inner diameter). A 1-μL extract was injected in splitless mode with the injector port temperature at 290 °C. Helium as carrier gas was maintained at a flow rate of 1.6 mL/min and nitrogen as collision gas at 1.5 mL/min. The oven temperature program was as follows: 70 °C for 4 min, then increased from 70 to 150 °C at 25 °C/min, 3 °C/min to 225 °C, and finally raised to 310 °C at 5 °C/min, where the temperature was held for 10 min. The MS/MS transfer line temperature was set at 250 °C.

Liquid chromatography–MS/MS. Metabolites were chromatographed by an Agilent 1200 liquid chromatograph (LC) interfaced to a 6410B triple-quadrupole MS/MS system (Agilent Technologies) with an electrospray ionization source in negative mode (N₂ 350 °C; gas flow 11 L/min; capillary 4000 V). Ten microliters of extract were injected and separated using a Luna 100A-C18 column (3 × 150 mm, 3 μm; Phenomenex) with a fritted disc (0.2 μm) heated at 40 °C at a flow of 0.5 L/min. The initial mobile phase was 0.1% formic acid in methanol:0.1% formic acid in m_qH₂O (70:30, v/v) for 5 min, followed by a 5-min gradient to 10% of 0.1% formic acid in m_qH₂O, a 3-min gradient to 0% of 0.1% formic acid in m_qH₂O, and a final 2 min to 30% of 0.1% formic acid in m_qH₂O.

Data acquisition

Data acquisition and quantification of the results were obtained using a MassHunter workstation software B.04.00 (Agilent Technologies). Peak detection and quantification were performed using a multiple reaction monitoring (MRM) and a dynamic MRM acquisition method for parent compounds and their metabolites, respectively. The MRM and dynamic MRM methods allow sensitive and precise quantitative analyses (Miller et al. 2010). For MRM conditions not provided by Sánchez-Avila et al. (2011) and Valton et al. (2014), acquisition parameters (retention times, parent ions, collision energies, quantifier and qualifier transitions) for 43 compounds (internal standards and analytes; marked by an asterisk in Supplemental Data, Tables S2 and S3) were individually optimized using Agilent Optimizer software. Transitions were selected following Sánchez-Avila et al. (2011), and MRM parameters for GC-MS/MS and LC-MS/MS are presented in Supplemental Data, Tables S2 and S3, respectively. Because of their incomplete separation, the respective peaks of the 2 isomeric monoesters MiBP and MnBP were identified by comparison of their retention times. The hydroxylated compounds of fluorene (2-OH-Flu, 3-OH-Flu, and 9-OH-Flu) and phenanthrene (1-OH-Phe, 2-OH-Phe, 3-OH-Phe, and 4-OH-Phe) were summed during peak integrations on MassHunter, quantified, and expressed as the sum of all isomers (i.e., OH-Flu and Oh-Phe) because of complex chromatographic separation. A linear regression analysis of the peak area ratio versus concentration ratio was

carried out to determine the concentration of each analyte. Quantification was performed by calculating the relative responses of each compound to the corresponding internal standard.

Method validation

Instrumental detection limit (IDL), limit of quantification (LOQ), recovery rate, repeatability, accuracy, and matrix effects were evaluated as stated in the European Union guidance document concerning the performance of analytical methods (European Commission 2002).

Linearity. The linearity of the calibration curve for parent compounds and metabolites was evaluated by regression analysis (determination coefficient, R^2) at the concentrations listed in Supplemental Data, Tables S4 and S5, which covered those found in our samples. For linearity criterion, a regression coefficient (R^2) higher than 0.98 was considered acceptable.

Sensitivity and procedural blank. With the less concentrated standard solution, IDLs were assessed at a signal-to-noise ratio (peak to peak) of 3. The LOQs were calculated as 9 times the signal-to-noise ratio using the spiked matrices. Five procedural blanks were performed to check for possible cross-contamination during laboratory procedures, following the same treatment steps as the samples. Following Laborie et al.'s, (2016) method, blanks were subtracted from sample quantifications when the concentration of the targeted compound was less than 4 times higher than the blank. When the concentration after blank correction was below the calculated LOQ value, the concentration was set to half the LOQ (Helsel 2006).

Recovery, repeatability, and accuracy. Recovery rates of parent compounds were assessed on replicate samples of chub muscle ($n = 7$) and liver ($n = 6$) with or without spiked solutions (i.e., to determine background levels of contamination of the procedure; Supplemental Data, Table S6). For metabolites, recovery experiments were performed on replicate chub liver ($n = 5$) and bile ($n = 5$) samples spiked at low and high levels (20 and 200 ng/g for liver samples, 20 and 200 ng/mL for bile samples). The repeatability of the method was assessed in terms of relative standard deviation (RSD) of the recovery. Syringe standards (benzyl benzoate, ¹³C₁₂PCB-194, and bromobiphenyl 209) were used to determine internal standard recoveries in muscle and liver samples for parent compounds (Supplemental Data, Table S7). To ensure data quality, certified reference materials were included in the method validation and matched well to our samples: EDF-2524 clean fish (Pacific herring, LGC Standards) for parent compounds and SRM-3672 human urine (LGC Standards) for metabolites. As the selected reference materials were not certified for all our targeted compounds, blank fish samples were spiked with native congeners.

Matrix effect. Because matrix components can coelute and alter the ionization of target analytes, matrix effects were calculated on GC and LC-MS/MS from replicate analyses ($n = 3$ for

bile, liver, and muscle samples) for parent compounds and metabolites, following Chen et al. (2012): Matrix effect (%) = $\frac{\text{Mean postextraction peak area}}{\text{Mean neat solution peak area}} \times 100$. A positive value of the matrix effect corresponds to a matrix-induced enhancement of analyte response, whereas a negative value corresponds to a suppression effect. Matrix effects were investigated at analyte concentrations of 100 ng/g in muscle and 200 ng/mL or ng/g in bile and liver samples, respectively.

Data presentation and statistical analysis

Concentrations of parent contaminants and their metabolites were expressed in muscle, liver, stomach content, gonad, and gill as ng/g of dry weight and ng/mL in bile. The relative contribution (percentage) to the total contamination is given as the sum of all congeners (i.e., Σ phthalates, Σ PAHs, Σ pyrethroids, Σ OCPs, Σ PCBs, and Σ PBDEs). Detection frequencies (percentage) of parent compounds and their metabolites were calculated in bile, liver, and muscle samples. Considering metabolites, the calculated LOQ values were highly dependent on the analyzed compounds. Thus, concentrations of metabolites below the LOQ were not included into the corresponding summed value (i.e., Σ phthalate metabolites, Σ PAH metabolites, Σ pyrethroid metabolites) to compare the contribution of each family of metabolites to the pollutant load in bile and liver samples.

RESULTS AND DISCUSSION

Method performance and validation

Chromatographic runs on GC- and LC-MS/MS. A total of 48 parent compounds and 20 of their metabolites were quantified within 59 and 15 min, respectively. The searched molecules were identified based on their selected transitions, retention time, and qualifier to quantifier ratios. Total ion chromatograms highlight the effectiveness of cleanup procedures with low or moderate (e.g., gonads) background noise (Figure 1 and Supplemental Data, Figure S1, for parent compounds and Figure 2 for metabolites) and the sensitivity of MS/MS. For each compound, highly linear responses were obtained over the concentration ranges tested (Supplemental Data, Tables S4 and S5). The coefficients of determination (R^2) were in the range 0.987 to 0.999 and 0.985 to 0.998 for parent compounds and their metabolites, respectively. Blank analyses ($n = 5$) constantly revealed the presence of phthalates (mean \pm SD, 165 ± 53.6 ng/g as Σ phthalates) and PAHs (17.1 ± 12.2 ng/g as Σ PAHs). Their ubiquity in the laboratory (materials, reagents) as well as their frequent detection in the indoor air (Abdel-Shafy and Mansour 2016) make analyses of environmental samples with low phthalate and PAH background contamination challenging (Fankhauser-Noti and Grob 2007) and explain the applied correction.

Sensitivity. The IDL values ranged from 0.01 to 13.6 and from 0.05 to 62.5 pg on column for parent compounds and metabolites, respectively, ensuring low-level detection of target analytes (Tables 2 and 3). Low LOQs (0.06–8.44 ng/g dry wt)

were determined for parent compounds and their by-products in solid matrices, although DEET, DiBP, and deltamethrin presented values higher than 12 ng/g dry weight. Of the 45 priority pollutants listed in the Water Framework Directive, 11 substances are set with environmental quality standards applicable to biota ($\text{EQS}_{\text{biota}}$; European Commission 2014). The present results are sufficient for compliance monitoring in biota samples because hazardous pollutants (benzo[a]pyrene, fluoranthene, HCB, PeCB, and DEHP) exhibited LOQs $\leq 30\%$ of their respective $\text{EQS}_{\text{biota}}$ (Table 2; Supplemental Data, Table S8). In bile samples, MMP, 3-OH-BaP, and 2-OH-Nap had LOQs > 5 ng/mL.

Recovery and accuracy. Acceptable recoveries (70–120%) were achieved for most of the selected internal standards in muscle and liver samples (Supplemental Data, Table S7) and allowed for reliable analyte quantification. Parent compounds exhibited satisfactory recoveries, with values ranging from 70 and 120%. However, naphthalene, phenothrin, p,p'-DDE, and some hydrocarbons of high molecular weight (chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[ghi]perylene) showed lower recoveries close to 50 to 60% (Table 2). These recoveries are in the acceptable range for these classes of contaminant as they are repeatable ($\text{RSD} \leq 20\%$) and in line with performance criteria set out for high molecular weight PAHs (recovery rate = 50–120%; European Commission 2011) and pesticide residues (recovery rate = 30–140%; European Commission 2017). The decreasing sensitivity of PAHs with increasing molecular weight is likely attributable to broader chromatographic peak and higher background noise commonly observed for molecules characterized by long retention times (Varlet et al. 2007; Figure 1). The use of specific labeled (deuterated or ^{13}C -enriched) internal standards should be considered to improve analytical performance for these compounds. Parent analytes displayed good repeatability (expressed as RSD) with values below the acceptable limit of 20%, except for 5 of them (acenaphthene, naphthalene, DEP, DnOP, and BDE-28) which might be explained by their volatility. Consequently, the method was not validated for naphthalene and DnOP, whose recoveries were unsatisfactory and presented poor precision (high RSD) in muscle and liver samples.

Most of the targeted metabolites exhibited satisfactory recoveries (70–120%) in bile and liver samples. A considerable increase in recovery was obtained for MBzP, MnOP, 3-PBA, and 4-FPBA as the concentration increased. However, MMP, MBzP, OH-Flu, and OH-Phe recoveries did not exceed 70% (Table 3) at low and high concentrations. With poor repeatability ($\text{RSD} \geq 20\%$) as well as low recovery, the method was therefore not reliable for these compounds. Molecules with the poorest recovery yields should be integrated into summed values of compounds within the same chemical family (e.g., Σ OH-PAHs) because they could still give us some information (detected or not, order of magnitude). Even if the method will underestimate the concentration of these compounds (low recovery), including them into summed values will limit biased results because they are the least detected in biota matrices (Ariese et al. 2005; Valton et al. 2014; Labadie et al. 2017).

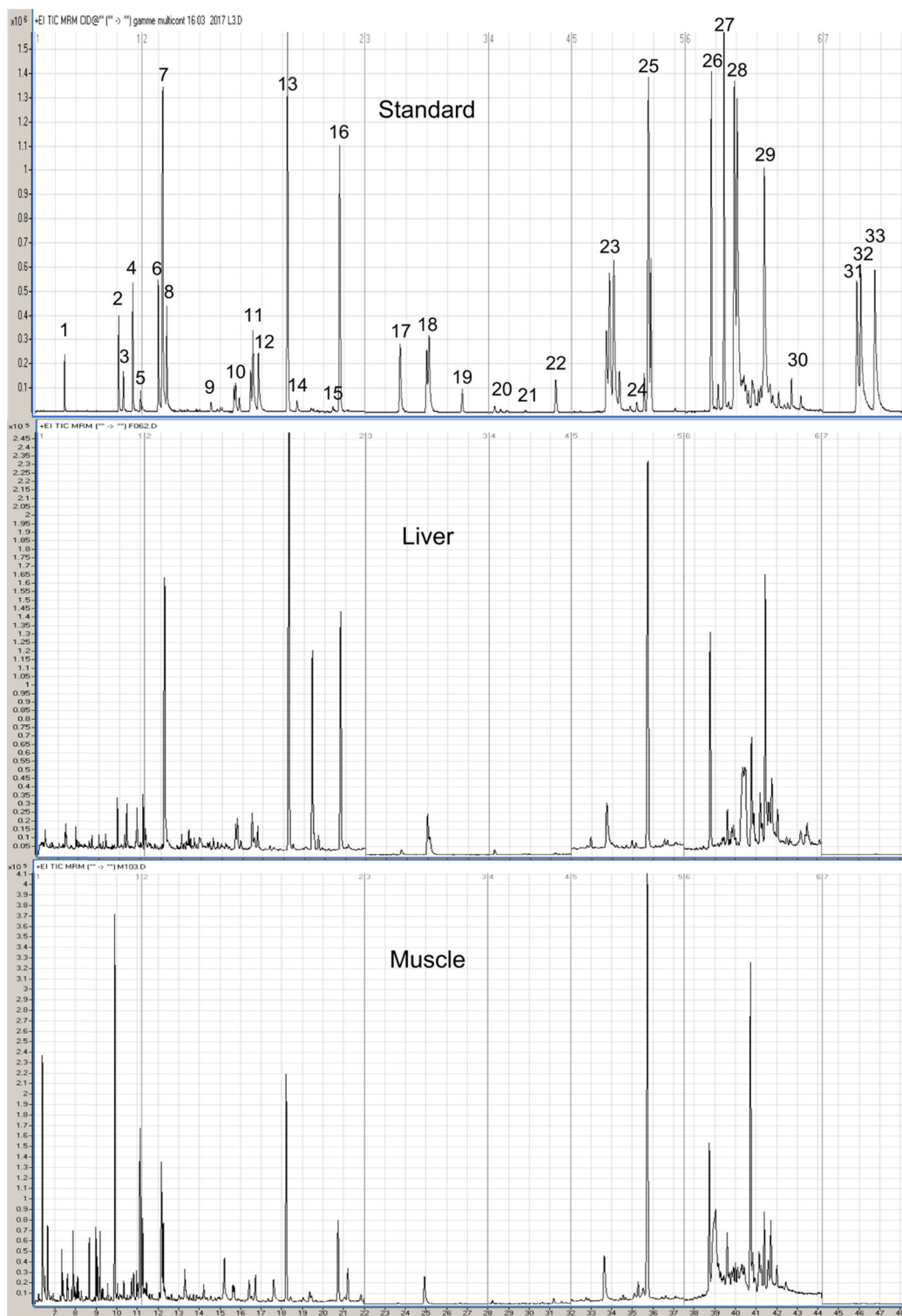


FIGURE 1: Total ion chromatogram of 48 parent compounds (phthalates, polycyclic aromatic hydrocarbons, organochlorine pesticides, polychlorinated biphenyls [PCBs], polybrominated diphenyl ethers, and insecticides: pyrethroids and *N,N*-diethyl-*meta*-toluamide [DEET]) under gas chromatography–tandem mass spectrometry conditions in a standard solution as well as in liver and muscle samples, obtained from MassHunter qualification software. The x- and y-axes represent the time (minutes) and relative abundance, respectively. Compounds are 1) naphthalene, 2) dimethyl phthalate, 3) acenaphthylene, 4) acenaphthene, 5) pentachlorobenzene, 6) DEET, 7) diethyl phthalate, 8) fluorene, 9) hexachlorobenzene, 10) lindane, 11) phenanthrene, 12) anthracene, 13) di-*iso*-decyl phthalate, 14) PCB-28, 15) PCB-52, 16) di-*n*-butyl phthalate, 17) fluoranthene, 18) pyrene, 19) dichlorodiphenyldichloroethylene, 20) PCB-101 + brominated diphenyl ether (BDE) 28, 21) PCB-153, 22) PCB-138 + *n*-butyl benzyl phthalate, 23) benzo[*a*]anthracene + chrysene + bifenthrin + PCB-118 + PCB-180, 24) BDE-47, 25) permethrin + di-2-ethylhexyl phthalate + phenothrin, 26) BDE-100, 27) di-*n*-octyl phthalate, 28) benzo[*b*]fluoranthene + benzo[*k*]fluoranthene + BDE-99 + cyfluthrin, 29) cypermethrin + benzo[*a*]pyrene, 30) fenvalerate + BDE-154 + deltamethrin + BDE-153, 31) indeno[1,2,3-*cd*]pyrene, 32) dibenzo[*a,h*]anthracene, 33) benzo[*g,h,i*]perylene. CID = collision-induced dissociation; EI = electron ionization; MRM = multiple reaction monitoring; TIC = total ion chromatogram.

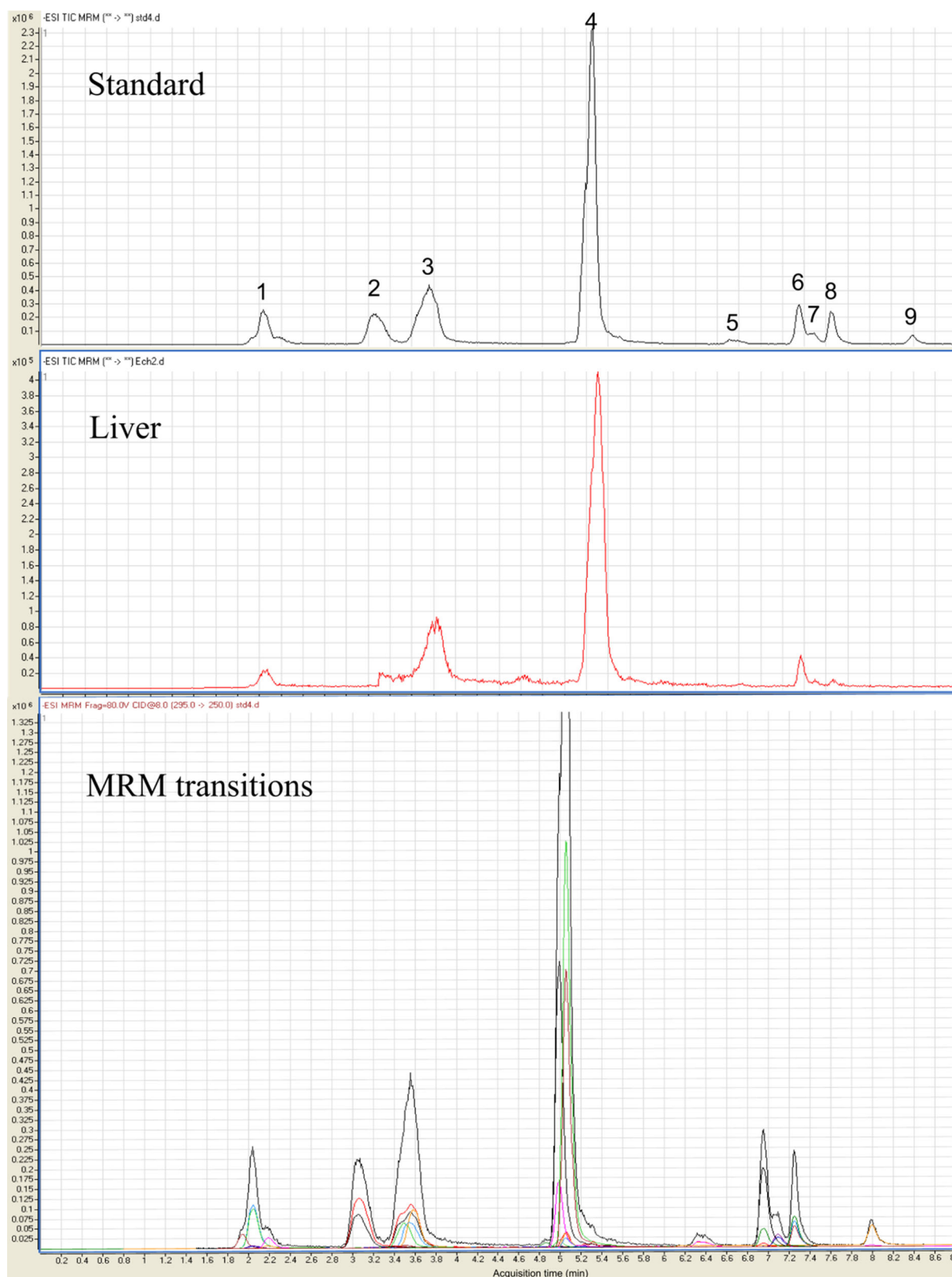


FIGURE 2: Total ion chromatogram of 20 metabolites under liquid chromatography–tandem mass spectrometry conditions in a standard solution and liver sample as well as the multiple reaction monitoring transitions, obtained from MassHunter qualification software. The x- and y-axes represent the time (minutes) and relative abundance, respectively. Compounds are 1) mono-methyl phthalate + mono-ethyl phthalate, 2) mono-2-ethyl-5-oxohexyl phthalate + 2-hydroxynaphthalene, 3) mono-benzyl phthalate + mono-iso-butyl phthalate + mono-2-ethyl-5-hydroxyhexyl phthalate + mono-*n*-butyl phthalate, 4) 4-fluoro-3-phenoxybenzoic acid + {*cis* + *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-1-cyclopropane} + 3-phenoxybenzoic acid + hydroxyfluorene + hydroxyphenanthrene, 5) hydroxypyrene, 6) mono-2-ethylhexyl phthalate, 7) 6-hydroxychrysene, 8) mono-*n*-octyl phthalate, 9) 3-hydroxybenzo[*a*]pyrene. CID = collision-induced dissociation; ESI = electrospray ionization; MRM = multiple reaction monitoring; TIC = total ion chromatogram.

TABLE 2: Parameters for validation of gas chromatography–tandem mass spectrometric method

Target compound	IDL (pg inj.) <i>n</i> = 3	Muscle (<i>n</i> = 7)			Liver (<i>n</i> = 6)		
		% RR	% RSD	LOQ (ng/g dry wt)	% RR	% RSD	LOQ (ng/g dry wt)
Acenaphthylene	0.04	103	7	0.94	88	15	1.93
Acenaphthene	0.05	93	5	0.88	77	22	1.93
Naphthalene	0.03	66	39	5.10	58	53	9.64
Anthracene	0.13	102	3	0.93	93	6	3.38
Fluorene	0.05	94	5	0.94	98	9	1.42
Phenanthrene	0.11	97	2	0.68	106	3	2.45
Fluoranthene	0.29	91	10	0.26	96	3	1.74
Pyrene	0.23	92	5	0.22	98	4	1.59
Benzo(a)anthracene	0.24	90	9	0.28	104	4	0.47
Chrysene	0.16	56	11	0.84	81	4	0.60
Benzo(a)pyrene	0.66	56	10	2.14	75	13	5.40
Benzo(b)fluoranthene	0.09	63	7	3.07	88	4	0.77
Benzo(k)fluoranthene	0.08	52	8	2.62	74	4	0.77
Benzo(g,h,i)perylene	0.08	48	8	0.35	99	4	1.08
Dibenzo(a,h)anthracene	0.10	69	6	0.43	105	6	0.60
Indeno(1,2,3-cd)pyrene	0.28	82	8	0.51	103	6	1.35
DMP	0.01	93	9	11.5	84	15	0.92
DEP	0.06	89	17	2.11	97	12	11.2
DiBP	0.04	132	31	2.72	97	19	30.00
DnBP	0.28	126	4	4.51	99	7	6.03
BBP	0.66	109	14	2.11	138	7	7.50
DEHP	0.02	125	9	1.29	75	4	7.71
DnOP	0.22	170	23	1.87	133	5	8.44
Bifenthrin	1.02	101	2	0.62	101	4	2.70
Permethrin	11.2	203	11	2.82	108	5	8.18
Phenothrin	10.2	66	3	6.63	113	5	3.00
Cyfluthrin	11.8	148	12	3.76	119	10	3.00
Cypermethrin	13.2	124	6	3.19	105	8	3.38
Fenvalerate	4.22	180	7	2.96	104	6	5.19
Deltamethrin	6.43	79	11	0.82	115	6	14.2
DEET	13.6	88	5	32.7	119	5	33.7
PeCB	0.02	86	8	0.53	94	13	0.90
HCB	0.06	78	4	0.20	101	7	0.51
Lindane	0.11	90	2	0.50	113	7	3.00
p,p'-DDE	0.02	94	11	0.18	64	11	2.63
PCB-28	0.01	92	7	0.24	112	4	0.48
PCB-52	0.03	93	5	0.09	102	4	0.25
PCB-101	0.03	103	5	0.18	121	3	2.45
PCB-153	0.06	87	6	0.32	90	4	0.18
PCB-138	0.08	95	14	0.32	90	4	0.16
PCB-118	0.19	84	17	1.98	96	3	2.25
PCB-180	0.11	100	19	1.98	98	3	0.83
BDE-28	0.05	72	46	0.06	100	4	2.65
BDE-47	0.13	151	7	0.12	100	4	0.38
BDE-100	0.32	127	4	0.26	75	3	3.00
BDE-99	0.32	144	3	0.22	92	5	2.87
BDE-154	0.54	94	5	0.32	74	4	1.96
BDE-153	0.87	143	3	0.35	112	5	1.69

BBP = *n*-butyl benzyl phthalate; BDE = brominated diphenyl ether; DEET = *N,N*-diethyl-*meta*-toluamide; DEHP = di-2-ethylhexyl phthalate; DEP = diethyl phthalate; DiBP = di-*iso*-butyl phthalate; DMP = di-*methyl* phthalate; DnBP, di-*n*-butyl phthalate; DnOP = di-*n*-octyl phthalate; HCB = hexachlorobenzene; IDL = instrumental detection limit; LOQ = limit of quantification; PCB = polychlorinated biphenyl; PeCB = pentachlorobenzene; p,p'-DDE = dichlorodiphenyldichloroethylene; RR = recovery rate; RSD = relative standard deviation.

The accuracy of the 2 methods was tested by analyzing reference materials EDF-2524 clean fish and SRM-3672 smoker's urine for parent compounds and metabolites, respectively (Supplemental Data, Tables S9 and S10). Our measurements were in good agreement with certified values, except for 3 high molecular weight PAHs (chrysene, benzo[*b*]fluoranthene and benzo[*k*]fluoranthene) and 2 phthalate metabolites (MEP and MEHHP) with *z* score absolute values outside the acceptable range ($|z \text{ score}| \geq 3$; Thompson et al. 2006).

Matrix effect. Matrix effects (percentage) for each analyte are presented in Supplemental Data, Table S11, for parent compounds. Our values in muscle samples ranged from 0% for phenanthrene to 92% for permethrin and were in line with the reported literature in fish matrices (Sapozhnikova and Lehotay 2013; Chatterjee et al. 2016). For parent compounds, 67% of the targeted analytes were reported, with matrix effects exceeding 20% in muscle against 50% of the analytes in liver samples. The considerable matrix effects for permethrin and cyfluthrin (92 and 36%

TABLE 3: Parameters for validation of the liquid chromatography–tandem mass spectrometric method

Target compound	IDL (pg inj.) <i>n</i> = 3	Liver				LOQ ng/g dw <i>n</i> = 10	Bile				LOQ ng/mL <i>n</i> = 10
		20 ng ^a , <i>n</i> = 5		200 ng ^a , <i>n</i> = 5			20 ng ^a , <i>n</i> = 5		200 ng ^a , <i>n</i> = 5		
		% RR	% RSD	% RR	% RSD		% RR	% RSD	% RR	% RSD	
MMP	0.92	62	21	40	60	10.0	68	36	37	9	13.3
MEP	2.14	76	20	73	10	11.7	74	26	67	17	2.99
MiBP	1.10	122	10	106	11	4.67	108	4	96	4	3.61
MEHHP	0.58	107	9	119	14	4.35	104	7	152	11	2.72
MnBP	1.05	102	9	93	10	5.07	91	3	103	6	4.90
MEOHP	0.47	109	11	264	41	1.47	94	10	151	11	0.95
MBzP	0.14	46	8	70	9	0.74	66	8	96	4	1.07
MEHP	2.37	77	16	86	7	11.8	93	6	104	4	0.94
MnOP	1.45	74	11	72	5	3.70	54	12	74	2	0.63
4-MU	0.98					–					–
2-OH-Nap	9.38	18	62	119	19	20.0	105	56	88	11	56.2
OH-Flu	2.25	52	27	31	78	4.86	74	33	80	42	3.80
OH-Phe	0.08	24	15	49	10	3.17	55	5	49	31	2.37
1-OH-Pyr	1.34	29	22	71	6	1.38	78	6	68	31	1.25
6-OH-Chr	4.50	49	8	78	10	2.26	91	13	88	13	2.92
3-OH-BaP	62.5	96	15	86	12	19.0	111	35	16	24	42.3
Cis-DCCA	1.31	102	10	91	5	106	79	7	95	6	2.33
Trans-DCCA	0.87	104	8	86	9	18.2	74	21	94	5	3.46
3-PBA	0.06	65	5	90	5	1.36	68	4	96	5	1.32
4-FPBA	0.05	52	10	75	5	1.21	62	2	86	5	1.22

^aSpiking level.

DCCA = 3-(2,2-dichlorovinyl)-2,2-dimethyl-1-cyclopropane; 4-FPBA = 4-fluoro-3-phenoxybenzoic acid; IDL = instrumental detection limit; LOQ = limit of quantification; MBzP = mono-benzyl phthalate; MEHHP = mono-2-ethyl-5-hydroxyhexyl phthalate; MEHP = mono-2-ethylhexyl phthalate; MEOHP = mono-2-ethyl-5-oxohexyl phthalate; MEP = mono-ethyl phthalate; MiBP = mono-iso-butyl phthalate; MMP = mono-methyl phthalate; MnBP = mono-*n*-butyl phthalate; MnOP = mono-*n*-octyl phthalate; 4-MU, 4-methylumbelliferone; 3-OH-BaP = 3-hydroxybenzo[*a*]pyrene; 6-OH-Chr = 6-hydroxychrysene; OH-Flu = hydroxyfluorene; 2-OH-Nap = 2-hydroxynaphthalene; OH-Phe = hydroxyphenanthrene; 1-OH-Pyr = 1-hydroxypyrene; 3-PBA = 3-phenoxybenzoic acid; RR = recovery rate; RSD = relative standard deviation.

in muscle, respectively) could explain their high recoveries (recovery rate >120%). In addition, matrix effects were highly variable between liver and muscle samples, which could explain the significant differences in terms of recovery and sensitivity of parent compounds in these matrices.

Higher matrix effects in terms of signal suppression were observed on LC-MS/MS than GC-MS/MS (Table 4) but were consistent with previous studies analyzing extracts of complex matrices (Ramsauer et al. 2010; Wang et al. 2017). Matrix effect in quantitative LC-MS/MS analyses of biological samples has been well documented (Smeraglia et al. 2002; Zhou et al. 2017), and protocols have been developed to minimize and manage them. To correct for these calculated matrix effects, specific internal standards were spiked in each measured fish sample with known quantities of analyte (see earlier section, *Matrix effect in Method validation*). Although matrix effects cannot be completely avoided in LC-MS/MS analyses, this method appears to minimize them because all the targeted metabolites in chub liver were reported with matrix effects exceeding 20% against 9 metabolites with internal standard addition. Similar results were observed in bile samples.

Organic pollutants in fish matrices

The methods were applied to the determination of the targeted analyte in several biological matrices of wild fish. Muscle and bile are commonly sampled in biomonitoring program (Kammann et al. 2014) to assess concentrations of parent compounds and their

by-products, respectively. Muscle is the most abundant tissue, less subjected to seasonal variation than other matrices (e.g., reproductive organs; Pollock 1984); and bile is an excretion medium in which hydroxylated and conjugated metabolites are predominantly eliminated (Meador et al. 1995). The application of our methods has been extended to other matrices because they can provide further information on pollutant load depending on the exposure pathway and physicochemical properties of toxicants: gills, because they are directly exposed to the aquatic environment; the liver, as a storage and metabolically active organ in which xenobiotics are transformed into more polar and excretable forms; stomach contents, for dietary exposure to pollutants; and gonads, as a storage and elimination pathway.

Concentrations of organic pollutants were found to vary inconsistently within individuals (Supplemental Data, Figure S2). Because only 3 females were analyzed, captured after the reproduction period in one hydrographic network, results could not be generalized. Among the 68 compounds quantified, 28 parent compounds and 5 metabolites were systematically found at detectable levels in the muscle, liver, and bile of chub (i.e., matrices with a calculated LOQ). Phenanthrene, DEHP, permethrin, *p,p'*-DDE, PCB-153, BDE-47, and MiBP were the most frequently detected substances of each contaminant family in the present study and previous ones (Xu et al. 2011; Valton et al. 2014; Couderc et al. 2015; Kampire et al. 2015). Because of the high hydrophobicity (K_{OW}) and low solubility of many organic contaminants, lipid contents are usually considered to explain chemical distributions in fish

TABLE 4: Calculated matrix effects for each metabolite analyte in bile and liver samples

Compound	Bile (n = 3)		Liver (n = 3)	
	ME (%)	Corrected ME (%) ^a	ME (%)	Corrected ME (%) ^a
MMP	-74	-57	-92	-53
MEP	-50	-18	-83	-2
MiBP	-28	-14	-83	32
MEHHP	19	17	-73	55
MnBP	36	27	-56	19
MEOHP	17	15	-45	17
MBzP	25	17	-66	-8
MEHP	-26	23	-83	-4
MnOP	-23	-8	-87	-4
2-OH-Nap	-14	18	-95	-39
OH-Flu	-34	-9	-92	-62
OH-Phe	-64	-50	-91	-59
1-OH-Pyr	96	-22	-83	-37
6-OH-Chr	-34	-9	-79	-20
3-OH-BaP	61	-36	-60	50
Cis-DCCA	4	16	-67	15
Trans-DCCA	4	16	-69	7
3-PBA	23	38	-75	24
4-FPBA	10	23	-75	11

^aMatrix effect corrected by internal standard (IS) addition.

DCCA = 3-(2,2-dichlorovinyl)-2,2-dimethyl-1-cyclopropane; 4-FPBA = 4-fluoro-3-phenoxybenzoic acid; MBzP = mono-benzyl phthalate; ME = matrix effect; MEHHP = mono-2-ethyl-5-hydroxyhexyl phthalate; MEHP = mono-2-ethylhexyl phthalate; MEOHP = mono-2-ethyl-5-oxohexyl phthalate; MEP = mono-ethyl phthalate; MiBP = mono-iso-butyl phthalate; MMP = mono-methyl phthalate; MnBP = mono-*n*-butyl phthalate; MnOP = mono-*n*-octyl phthalate; 3-OH-BaP = 3-hydroxybenzo[a]pyrene; 6-OH-Chr = 6-hydroxychrysene; OH-Flu = hydroxyfluorene; 2-OH-Nap = 2-hydroxynaphthalene; OH-Phe = hydroxyphenanthrene; 1-OH-Pyr = 1-hydroxypyrene; 3-PBA = 3-phenoxybenzoic acid.

matrices (Kampire et al. 2015). The highest lipid contents were measured in stomach content and gill samples, followed by liver and, to a lesser extent, muscles and gonads (Table 1). Gonads (1477–10 028 ng/g dry wt, Σ parent compounds) and stomach contents (1809–6701 ng/g dry wt) were the most contaminated matrices (Figure 3A), followed by liver (712–4542 ng/g dry wt) > gill (899–1127 ng/g dry wt) > muscle (338–529 ng/g dry wt). Here, lipid contents alone could not explain the strong discrepancies of organic contaminants in chub matrices. Indeed, pollutant distributions throughout the fish body are generally governed by complex mechanisms including the analyte susceptibility to metabolic biotransformation (K_m) and its physicochemical properties (molecular weight and K_{OW}). Although gills are in direct contact with the aquatic environment (i.e., gill–water transfer), organic pollutant concentrations were 2 to 4 times lower than in stomach content, gonad, and liver samples. This is not surprising given the moderate hydrophobicity of most target analytes ($\log K_{OW} > 5$). As stated in previous studies, low levels of contaminants were detected in fish muscle, demonstrating that it is not the most relevant tissue to sample regarding the accumulation of organic compounds (Zhang et al. 2010; Xu et al. 2011; Teil et al. 2012).

Parent compounds in chub matrices. Phthalates and pyrethroids were the most abundant metabolizable pollutants in chub. As far as we know, this is the second study aiming at quantifying pyrethroid pesticides in wild fish (Corcellas et al. 2015). As sampling differs (whole fish vs fish organs), further comparison (concentration and pattern distribution of each pyrethroid) could

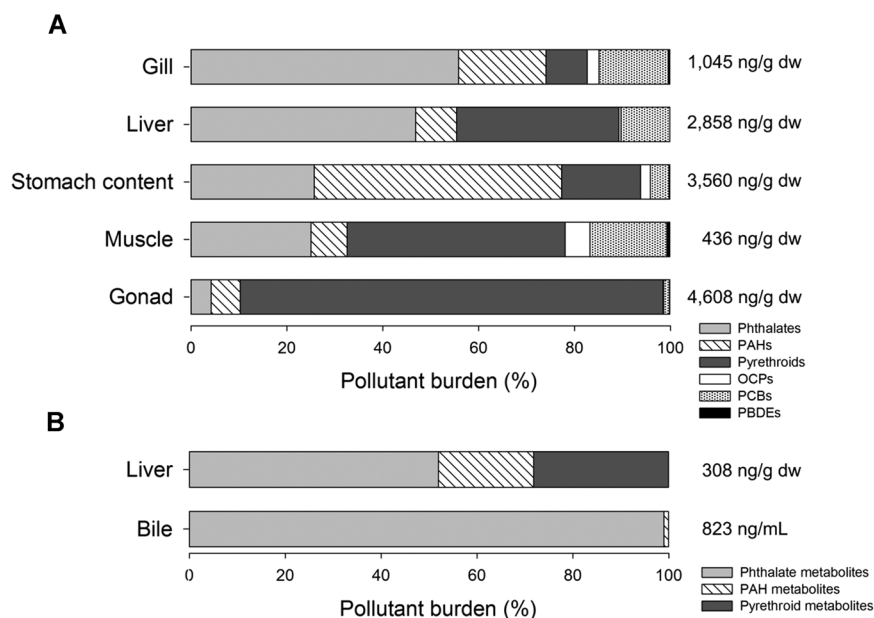


FIGURE 3: Relative contribution (percentage) of (A) parent compounds (phthalates, polycyclic aromatic hydrocarbons, pyrethroids, organochlorine pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers) and (B) their metabolites in all investigated chub (n = 3) matrices. Mean contamination is expressed as nanograms per gram of dry weight for gill, liver, stomach content, muscle, and gonad samples and as nanograms per milliliter for bile samples. OCP = organochlorine pesticide; PAH = polycyclic aromatic hydrocarbon; PBDE = polybrominated diphenyl ether; PCB = polychlorinated biphenyl.

not be achieved. A greater proportion of PAHs was measured in stomach contents compared to the remaining matrices (Figure 3A). This result suggests that the primary source of PAHs in fish is the consumption of contaminated food or the ingestion of PAHs adsorbed onto the particulate phase and sediments. The distribution of phthalates matches findings in Teil et al. (2012) because the liver was the most contaminated chub tissue (191–3070 ng/g dry wt), followed by gonads (106–237 ng/g dry wt) and muscles (57–72 ng/g dry wt). Interestingly, levels of metabolizable pollutants were 3 to 63 times higher than those of persistent organic pollutants (POPs). Despite their rapid biodegradation, metabolizable pollutants behave as persistent chemicals (pseudo-persistence) because they are continually infused to the aquatic environment. Conversely, most legacy POPs are subject to regulations, and their levels have been declining over the last decades (Rig  t et al. 2019). Persistent organic pollutants were characterized by a higher proportion of Σ PCBs (20.27–657 ng/g dry wt) and, to a lesser extent, by Σ OCPs (3.14–158 ng/g dry wt) and Σ PBDEs (1.23–12.51 ng/g dry wt). In liver samples, PCB concentrations were over 4 times higher than those in muscle. This result is in line with the scientific literature because the liver accumulated higher levels of PCBs than any other organ (Kr  a et al. 2007; Br  zov   et al. 2012). Overall, the distribution patterns of legacy POPs throughout the fish body (Figure 3A) were in good agreement with previous work (Teil et al. 2012; Kampire et al. 2015).

Metabolites in fish matrices. Phthalate monoesters represented up to 99% of the pollutant load (Figure 3B) in bile samples and were the most frequently detected. Lower detection frequencies were reported for PAHs and pyrethroid metabolites, with only 1-OH-Pyr detected in bile samples. Levels of metabolites were comparable to those of parent compounds within chub liver, which strengthens the usefulness of metabolite analyses to better understand body burdens of organic pollutants in wild fish. In addition, the contributions of phthalates, PAHs, and pyrethroid metabolites to the total load were consistent with those reported for their parent compounds in chub liver (Σ phthalates > Σ pyrethroid pesticides > Σ PAHs; Figure 3A). Pyrethroid metabolites were quantified for the first time in wild organisms and only *cis*- and *trans*-DCCA were detected in liver samples above their LOQs. In bile and liver samples, MiBP and 1-OH-Pyr were typically the dominant metabolites. Similar trends have been reported in various fish species caught in offshore (Tomy et al. 2014), coastal (Vuorinen et al. 2017), and urban (Valton et al. 2014) areas. Because these metabolites have been poorly characterized in freshwater fish, with only 1-OH-Pyr and 1-OH-Phe mentioned for environmental monitoring in European guidelines (Helsinki Commission 2013), few comparisons (e.g., concentration range, congener profiles) with previous studies were possible because they investigated diverse matrices and species from different geographical regions, resulting in highly variable results (Kammann et al. 2014; Ros et al. 2015; Fourgous et al. 2016).

CONCLUSION

These 2 methods were developed to determine 16 PAHs, 7 phthalates, 12 pesticides, 7 PCBs, and 6 PBDEs as well as 20 of

their metabolites in fish matrices at environmentally relevant concentrations. With performance parameters meeting the validation criteria for most of the targeted compounds, the methods were then successfully applied for reliable characterization of organic pollutants in wild fish. These analytical protocols represent a new and suitable procedure, requiring small amounts of biological samples, for the quantification of a broad spectrum of molecules in complex matrices. With few adjustments, additional hazardous compounds could be incorporated in our developed methods under the operating MRM mode.

Whatever the investigated matrix, a higher proportion of metabolizable pollutants versus persistent pollutants was detected. Overall, pyrethroids and plasticizers were the prevailing organic compounds, with gonads and stomach contents being the most contaminated matrices. Even though fish muscle and biliary fluid are a practical and suitable option to assess contaminant exposure, other organs (e.g., liver) should be considered to detect a wider range of analytes at higher concentrations. Because metabolizable pollutants are rapidly transformed into more polar and excretable products, the quantification of both parent compounds and metabolites gives a comprehensive overview of the pollutant load in fish. Indeed, parent compounds in fish muscles and metabolites in liver samples were within the same order of magnitude. Thus, organisms might be more at risk than is currently perceived in bio-monitoring programs because metabolites of xenobiotics could present a similar or even higher toxicity level. Metabolite measurements should be used as a complementary tool for a more comprehensive assessment of the environmental contamination and the ecological risks for aquatic organisms.

Supplemental Data—Supplemental data are available on the Wiley Online Library at DOI: 10.1002/etc.4500.

Acknowledgment—Funding was provided by the Sorbonne University Environmental Transition Institute. The present study was financially supported by the Seine-Normandy Water Agency (research program 1059502). The authors are very grateful to A. Desportes and C. Bourges for their technical assistance.

Data Accessibility—Data are available from the corresponding author (aurelie.goutte@upmc.fr).

REFERENCES

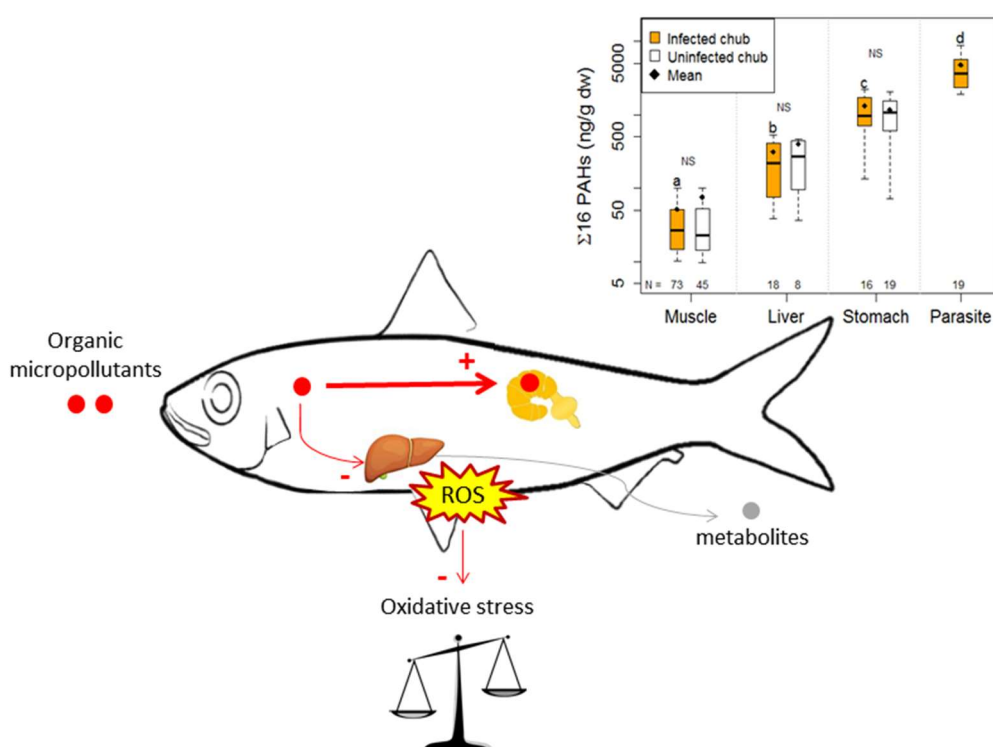
- Abdel-Shafy HI, Mansour MSM. 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum* 25:107–123.
- Ariese F, Beyer J, Jonsson G, Porte C, Krahn MM. 2005. Review of analytical methods for determining metabolites of polycyclic aromatic compounds (PACs) in fish bile. ICES Techniques in Marine Environmental Sciences 39. International Council for the Exploration of the Sea, Copenhagen, Denmark.
- Barron MG, Albro PW, Hayton WL. 1995. Biotransformation of di(2-ethylhexyl) phthalate by rainbow trout. *Environ Toxicol Chem* 14:873–876.
- Br  zov   T, Hanzelov   V, Miklisov   D. 2012. Bioaccumulation of six PCB indicator congeners in a heavily polluted water reservoir in eastern Slovakia: Tissue-specific distribution in fish and their parasites. *Parasitol Res* 111:779–786.
- Carpenter DO. 2011. Health effects of persistent organic pollutants: The challenge for the Pacific basin and for the world. *Rev Environ Health* 26:61–69.

- Chatterjee NS, Utture S, Banerjee K, Shabeer A, Kamble N, Mathew S, Kumar KA. 2016. Multiresidue analysis of multiclass pesticides and polyaromatic hydrocarbons in fatty fish by gas chromatography tandem mass spectrometry and evaluation of matrix effect. *Food Chem* 196:1–8.
- Chen M, Tao L, Collins EM, Austin C, Lu C. 2012. Simultaneous determination of multiple phthalate metabolites and bisphenol-A in human urine by liquid chromatography–tandem mass spectrometry. *J Chromatogr B* 904:73–80.
- Corcellas C, Eljarrat E, Barceló D. 2015. First report of pyrethroid bioaccumulation in wild river fish: A case study in Iberian river basins (Spain). *Environ Int* 75:110–116.
- Couderc M, Poirier L, Zalouk-Vergnoux A, Kamari A, Blanchet-Letrouvé I, Marchand P, Vénisseau A, Veyrand B, Mouneyrac C, Le Bizec B. 2015. Occurrence of POPs and other persistent organic contaminants in the European eel (*Anguilla anguilla*) from the Loire estuary, France. *Sci Total Environ* 505:199–215.
- European Commission. 2002. Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official J Eur Union* L221:8–36.
- European Commission. 2014. Common Implementation Strategy for the Water Framework Directive (2000/60/EC): Guidance document no. 32 on biota monitoring (the implementation of EQS Biota) under the Water Framework Directive. Technical report. Luxembourg.
- European Commission. 2017. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. Technical report SANTE/11813/2017. Brussels, Belgium.
- European Commission. 2011. Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. *Official J Eur Union* L215:9–16.
- European Environment Agency. 2019. Chemicals in European waters: Knowledge developments. Technical report 18. Copenhagen, Denmark.
- Fankhauser-Noti A, Grob K. 2007. Blank problems in trace analysis of diethylhexyl and dibutyl phthalate: Investigation of the sources, tips and tricks. *Anal Chim Acta* 582:353–360.
- Fourgous C, Chevreuil M, Alliot F, Amilhat E, Faliex E, Paris-Palacios S, Teil MJ, Goutte A. 2016. Phthalate metabolites in the European eel (*Anguilla anguilla*) from Mediterranean coastal lagoons. *Sci Total Environ* 569–570:1053–1059.
- Frederiksen H, Jørgensen N, Andersson A-M. 2010. Correlations between phthalate metabolites in urine, serum, and seminal plasma from young Danish men determined by isotope dilution liquid chromatography tandem mass spectrometry. *J Anal Toxicol* 34:400–410.
- Hájková K, Pulkrabová J, Hajšlová J, Randák T, Žlábek V. 2007. Chub (*Leuciscus cephalus*) as a bioindicator of contamination of the Vltava River by synthetic musk fragrances. *Arch Environ Contam Toxicol* 53:390–396.
- Hamilton PB, Cowx IG, Oleksiak MF, Griffiths AM, Grahn M, Stevens JR, Carvalho GR, Nicol E, Tyler CR. 2016. Population-level consequences for wild fish exposed to sublethal concentrations of chemicals—A critical review. *Fish Fish (Oxf)* 17:545–566.
- Helsinki Commission. 2013. HELCOM core indicators: Final report of the HELCOM CORESET project. Baltic Sea Environment Proceedings 136. Helsinki, Finland.
- Helsel DR. 2006. Fabricating data: How substituting values for nondetects can ruin results, and what can be done about it. *Chemosphere* 65:2434–2439.
- Kammann U, Brinkmann M, Freese M, Pohlmann J-D, Stoffels S, Hollert H, Hanel R. 2014. PAH metabolites, GST and EROD in European eel (*Anguilla anguilla*) as possible indicators for eel habitat quality in German rivers. *Environ Sci Pollut Res* 21:2519–2530.
- Kampire E, Rubidge G, Adams JB. 2015. Distribution of polychlorinated biphenyl residues in several tissues of fish from the North End Lake, Port Elizabeth, South Africa. *Water SA* 41:559–570.
- Krča S, Žaja R, Čalić V, Terzić S, Grubešić MS, Ahel M, Smilaj T. 2007. Hepatic biomarker responses to organic contaminants in feral chub (*Leuciscus cephalus*)—Laboratory characterization and field study in the Sava River, Croatia. *Environ Toxicol Chem* 26:2620–2633.
- Labadie P, Alliot F, Bourges C, Desportes A, Chevreuil M. 2010. Determination of polybrominated diphenyl ethers in fish tissues by matrix solid-phase dispersion and gas chromatography coupled to triple quadrupole mass spectrometry: Case study on European eel (*Anguilla anguilla*) from Mediterranean coastal lagoons. *Anal Chim Acta* 675:97–105.
- Labadie P, Simonnet-Laprade C, Maciejewski K, Lachaux V, Le Menach K, Budzinski H, Alliot F, Chevreuil M, Santos R, Goutte A. 2017. Transfers de micropolluants dans les réseaux trophiques lotiques. Scientific Report. PIREN-Seine phase VII. Paris, France.
- Laborie S, Moreau-Guigon E, Alliot F, Desportes A, Oziol L, Chevreuil M. 2016. A new analytical protocol for the determination of 62 endocrine-disrupting compounds in indoor air. *Talanta* 147:132–141.
- Malaj E, von der Ohe PC, Grote M, Kühne R, Mondy CP, Usseglio-Polatera P, Brack W, Schäfer RB. 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc Natl Acad Sci USA* 111:9549–9554.
- Meador JP, Stein JE, Reichert WL, Varanasi U. 1995. Bioaccumulation of polycyclic aromatic hydrocarbons by marine organisms. *Rev Environ Contam Toxicol* 143:79–165.
- Miller C, Waddell K, Tang N. 2010. High throughput protein quantitation using MRM viewer software and dynamic MRM on a triple quadrupole mass spectrometer. *J Biomol Tech* 21(3 Suppl.):S60.
- Nagyová S, Tölgyessy P. 2019. Validation including uncertainty estimation of a GC-MS/MS method for determination of selected halogenated priority substances in fish using rapid and efficient lipid removing sample preparation. *Foods* 8:101.
- Ondarza PM, Gonzalez M, Fillmann G, Miglioranza KSB. 2014. PBDEs, PCBs and organochlorine pesticides distribution in edible fish from Negro River basin, Argentinean Patagonia. *Chemosphere* 94:135–142.
- Pollock BR. 1984. Relations between migration, reproduction and nutrition in yellowfin bream *Acanthopagrus australis*. *Mar Ecol Prog Ser* 19:17–23.
- Ramsauer B, Sterz K, Hagedorn HW, Engl J, Scherer G, McEwan M, Errington G, Shepperd J, Cheung F. 2010. A liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of phenolic polycyclic aromatic hydrocarbons (OH-PAH) in urine of non-smokers and smokers. *Anal Bioanal Chem* 399:877–889.
- Rigét F, Bignert A, Braune B, Dam M, Dietz R, Evans M, Green N, Gunnlaugsdóttir H, Hoydal KS, Kucklick J, Letcher R, Muir D, Schuur S, Sonne C, Stern G, Tomy G, Wilson S. 2019. Temporal trends of persistent organic pollutants in Arctic marine and freshwater biota. *Sci Total Environ* 649:99–110.
- Ros O, Izaguirre JK, Olivares M, Bizarro C, Ortiz-Zarragoitia M, Cajaraville MP, Etxebarria N, Prieto A, Vallejo A. 2015. Determination of endocrine disrupting compounds and their metabolites in fish bile. *Sci Total Environ* 536:261–267.
- Sánchez-Avila J, Fernandez-Sanjuan M, Vicente J, Lacorte S. 2011. Development of a multi-residue method for the determination of organic micropollutants in water, sediment and mussels using gas chromatography–tandem mass spectrometry. *J Chromatogr A* 1218:6799–6811.
- Sapozhnikova Y, Lehotay SJ. 2013. Multi-class, multi-residue analysis of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and novel flame retardants in fish using fast, low-pressure gas chromatography–tandem mass spectrometry. *Anal Chim Acta* 758:80–92.
- Smeraglia J, Baldrey SF, Watson D. 2002. Matrix effects and selectivity issues in LC-MS-MS. *Chromatographia* 55(Suppl. 1):S95–S99.
- Teil M-J, Tlili K, Blanchard M, Chevreuil M, Alliot F, Labadie P. 2012. Occurrence of polybrominated diphenyl ethers, polychlorinated biphenyls, and phthalates in freshwater fish from the Orge River (Ile-de France). *Arch Environ Contam Toxicol* 63:101–113.
- Thompson M, Ellison SLR, Wood R. 2006. The international harmonized protocol for the proficiency of analytical chemistry laboratories (IUPAC Technical Report). *Pure Appl Chem* 78:145–196.
- Tomy GT, Halldorson T, Chernomas G, Bestvater L, Danegerfield K, Ward T, Gtskch K, Stern G, Atchison S, Majewski A, Reist JD, Palace VP. 2014. Polycyclic aromatic hydrocarbon metabolites in arctic cod (*Boreogadus saida*) from the Beaufort Sea and associative fish health effects. *Environ Sci Technol* 48:11629–11636.
- Valton AS, Serre-Dargnat C, Blanchard M, Alliot F, Chevreuil M, Teil MJ. 2014. Determination of phthalates and their by-products in tissues of roach (*Rutilus rutilus*) from the Orge River (France). *Environ Sci Pollut Res* 21:12723–12730.
- Van der Oost R, Beyer J, Vermeulen NP. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environ Toxicol Pharmacol* 13:57–149.

- Varlet V, Serot T, Monteau F, Le Bizec B, Prost C. 2007. Determination of PAH profiles by GC-MS/MS in salmon processed by four cold-smoking techniques. *Food Addit Contam* 24:744–757.
- Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies PM. 2010. Global threats to human water security and river biodiversity. *Nature* 467:555–561.
- Vuorinen PJ, Saulamo K, Lecklin T, Rahikainen M, Koivisto P, Keinänen M. 2017. Baseline concentrations of biliary PAH metabolites in perch (*Perca fluviatilis*) in the open Gulf of Finland and in two coastal areas. *J Mar Syst* 171:134–140.
- Wang Y, Meng L, Pittman EN, Etheredge A, Hubbard K, Trinidad DA, Kato K, Ye X, Calafat AM. 2017. Quantification of urinary mono-hydroxylated metabolites of polycyclic aromatic hydrocarbons by on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 409:931–937.
- Xu F-L, Wu W-J, Wang J-J, Qin N, Wang Y, He Q-S, He W, Tao S. 2011. Residual levels and health risk of polycyclic aromatic hydrocarbons in freshwater fishes from Lake Small Bai-Yang-Dian, northern China. *Ecol Modell* 222:275–286.
- Zhou W, Yang S, Wang PG. 2017. Matrix effects and application of matrix effect factor. *Bioanalysis* 9:1839–1844.
- Zhang K, Wan Y, Giesy JP, Lam MHW, Wiseman S, Jones PD, Hu J. 2010. Tissue concentrations of polybrominated compounds in Chinese sturgeon (*Acipenser sinensis*): Origin, hepatic sequestration, and maternal transfer. *Environ Sci Technol* 44:5781–5786.
- Zhang X, Hou H, Xiong W, Hu Q. 2015. Development of a method to detect three monohydroxylated polycyclic aromatic hydrocarbons in human urine by liquid chromatographic tandem mass spectrometry. *J Anal Methods Chem* 2015:1–8.

Article 2

Molbert N, Alliot A, Leroux-Coyau M, Médoc V, Biard C, Meylan S, Jacquin L, Santos R, Goutte A. 2020 Potential benefits of acanthocephalan parasites for chub hosts in polluted environments. *Environ. Sci. Technol.* (doi:10.1021/acs.est.0c00177)



Potential Benefits of Acanthocephalan Parasites for Chub Hosts in Polluted Environments

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Cite This: <https://dx.doi.org/10.1021/acs.est.0c00177>



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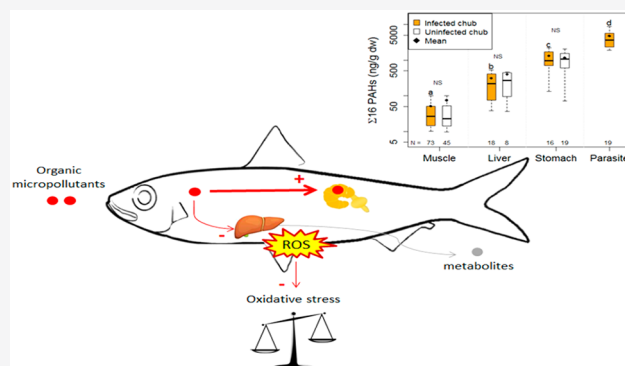


Article Recommendations



Supporting Information

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 biotic¹ and environmental^{2–4} conditions. Among the multiple factors investigated, the effect of chemical pollution on species interaction remains poorly investigated.⁵ 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.⁶).

Acanthocephalan parasites are endoparasitic worms that are transmitted from an arthropod intermediate host to the intestine of a vertebrate definitive host.⁷ These parasites are known to negatively alter the physiology of fish⁸ and influence life-history traits such as reproduction⁹ and behavior.¹⁰ On the other hand, acanthocephalan parasites could act as toxicant sinks and reduce the pollutant load of their host.¹¹ Intestinal parasites, particularly *Pomphorhynchus* sp., are able to concentrate environmental pollutants, sometimes at orders of magnitude higher than in their host tissues.^{12–15} Up to now, studies have mainly focused on trace metals and more recently on polychlorinated biphenyls (PCBs),¹⁶ a family of persistent

pollutants no longer produced or used. To date, very few studies have considered the effects of emerging organic pollutants^{17,18} 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.¹⁹ In addition, their metabolization leads to the formation of toxic byproducts that are sometimes even more noxious than the parent pollutants.²⁰

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

Received: January 9, 2020

Revised: March 5, 2020

Accepted: April 8, 2020

Published: April 8, 2020

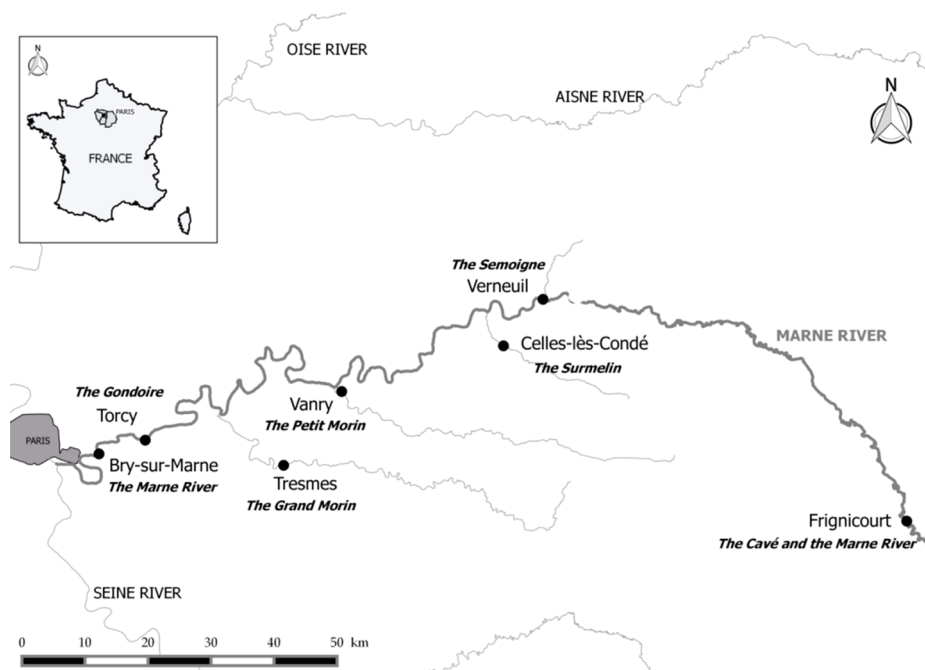


Figure 1. Sampling sites on the Marne River (France) and its tributaries (Created using QGIS 3.4.12 software on French geoportal IGN-F layers).

vary with the trophic level, length/age, and lipid contents for hydrophobic molecules (i.e., octanol–water partition coefficient: $\log K_{OW}$).^{21,22} Second, vertebrates and invertebrates differ strongly in their ability to metabolize xenobiotics,²³ 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.²⁴ Acanthocephalan parasites lack a gastrointestinal tract, so that food assimilation mainly occurs through passive mechanisms.²⁵ This physiological trait is likely to favor smaller and more water-soluble molecules,^{26,24} 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,²⁷ body conditions,²⁸ and enhanced oxidative stress.²⁹ On the other hand, cestode infection has been shown to reduce intermediate host mortality across a range of arsenic concentrations.³⁰ Similarly, decreased survival with increasing lead (Pb) levels was observed in common eiders (*Somateria mollissima*) treated with an antiparasite drug,⁵ suggesting a protective effect of parasites in Pb-exposed individuals. General biomarkers are known to respond to a variety of stressors, natural or anthropogenic,³¹ 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.³² 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,^{33,34} 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.³⁵ Yet, studies have reported contrasting results on the joint effect of pollution and parasitism on immunity.^{36,37}

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,³⁸ thus impacting their energetic status. In addition, parasites can have neutral, negative, or positive effects on the host body condition,^{39–41} by depleting host resources or by enhancing host lipid content.³⁰ 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.⁴² Living up to 20 years and tolerant to pollution, chub are good ecotoxicological models prone to accumulate toxicants.⁴³ 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:⁴⁴ 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.⁴⁵

Blood was collected from sedated fish (tricaine methanesulfonate: MS222, 80 mg·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 , ± 0.1 cm) and body weight (W_T , ± 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 , ± 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.⁴⁶ Biological samples were stored in polycarbonate tubes to limit phthalate contamination and frozen at -20 °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.⁴⁷ 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.⁴⁸ 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 Roth⁴⁹ at Bordeaux Sciences Agro (Gradignan, France). Briefly, a 96-well plate was filled with 5 μL of fish plasma in 400 μL of HBBS (Hank's balanced salt solution) buffer. The reaction mixture was added to the microplate and consisted of 100 μL of NaH_2PO_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 μL of sulfuric acid (H_2SO_4 , 2 M). The absorbance was recorded at 450 nm.

Following the procedure described by Ellis,⁵⁰ lysozyme activity was measured using fish plasma (15 μL) and 150 μL of a solution of *Micrococcus lysodeikticus* suspension (20 mg 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,⁵¹ which derive from the oxidation of biomolecular substrates and act as precursors of end-products of lipid peroxidation.⁵² 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.,⁵³ using 4 μL (d-ROMs) and 5 μL (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 H_2O_2 equivalents.

Chemical Analysis and Lipid Contents. The analytical methods used are described in the work of Molbert et al.⁴⁴ 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^{-1} of dry wt) in *Pomphorhynchus* sp. Relative to That in Host Muscle ($n = 19$), Liver ($n = 9$), and Stomach Content ($n = 12$)^a

compounds	$C_{[\text{parasite}]} / C_{[\text{chub muscle}]}$	$C_{[\text{parasite}]} / C_{[\text{chub liver}]}$	$C_{[\text{parasite}]} / C_{[\text{chub stomach}]}$
	mean \pm SD	mean \pm SD	mean \pm SD
$\sum 7$ phthalates	22.8 \pm 32.2 (***)	8.45 \pm 7.73 (*)	7.71 \pm 12.7 (*)
$\sum 16$ PAHs	211 \pm 187 (***)	86.2 \pm 124 (**)	3.40 \pm 1.50 (***)
$\sum 7$ pyrethroids	1.51 \pm 2.14	1.93 \pm 2.99	1.94 \pm 2.75
DEET	1.98 \pm 0.99 (**)	2.05 \pm 1.11 (*)	0.66 \pm 0.91
$\sum 4$ OCPs	6.60 \pm 7.46 (***)	2.16 \pm 1.00 (**)	0.43 \pm 0.52 (*)
$\sum 7$ PCBs	3.05 \pm 1.86 (***)	1.98 \pm 1.48 (*)	1.15 \pm 0.77
$\sum 6$ PBDEs	3.39 \pm 2.44 (***)	0.76 \pm 0.53	1.03 \pm 0.86

^aPAH = 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.⁵⁴ 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 \pm standard deviation (SD). Contaminant levels were not related to lipid content (all $p > 0.05$). Consequently, the levels of contaminants (\sum phthalates, \sum PAHs, \sum pyrethroids, \sum OCPs, \sum PCBs, \sum PBDEs, \sum phthalate metabolites, \sum hydroxy-PAHs, and \sum pyrethroid metabolites) are expressed in nanograms per gram of dry weight (see the work of Hebert and Keenleyside⁵⁵). Data were analyzed using linear mixed models (LMMs) with the *lme4* package⁵⁶ in R v. 3.3.2 software.⁵⁷ The *lmerTest* package provided p -values via Satterthwaite's degrees of freedom method.⁵⁸ 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 ($r = C_{[\text{Parasite}]} / C_{[\text{chub matrix}]}$)⁵⁹ 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: $\log K_{\text{OW}}$, metabolic rate: $\log k_{\text{M}}$, and molecular weight), which were extracted from the EPA's EPI software suite (EPIWEB 4.1 package).⁶⁰

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 collinearity 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.⁶¹

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 \pm SD [range]) of fish matrices were the following: muscle 3.01 ± 1.72 [0.56–8.98]%, liver 6.19 ± 3.99 [1.59–14.5]%, and stomach content (8.76 ± 4.77 [3.35–19.8]%). There were no significant differences in lipid content between acanthocephalan parasites (4.71 ± 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 \sum 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 ($F_{(1,60)} = 6.00$, $p =$

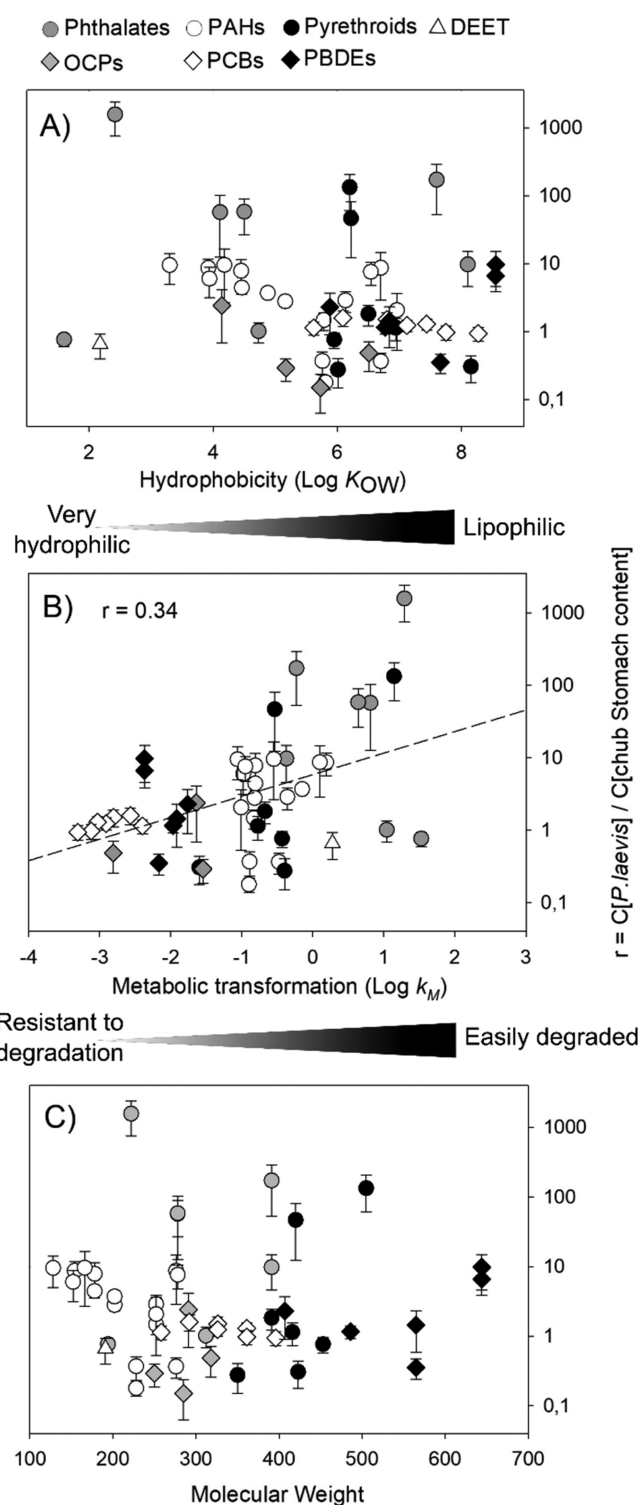


Figure 2. Bioaccumulation ratio (r ; mean \pm SD) of 48 organic pollutants (ng g^{-1} of dry wt) in *Pomphorhynchus* sp. relative to fish stomach content in relation to (A) their hydrophobicity ($\log K_{OW}$), (B) metabolic biotransformation ($\log k_M$), and (C) molecular weight [PAHs = polycyclic 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:

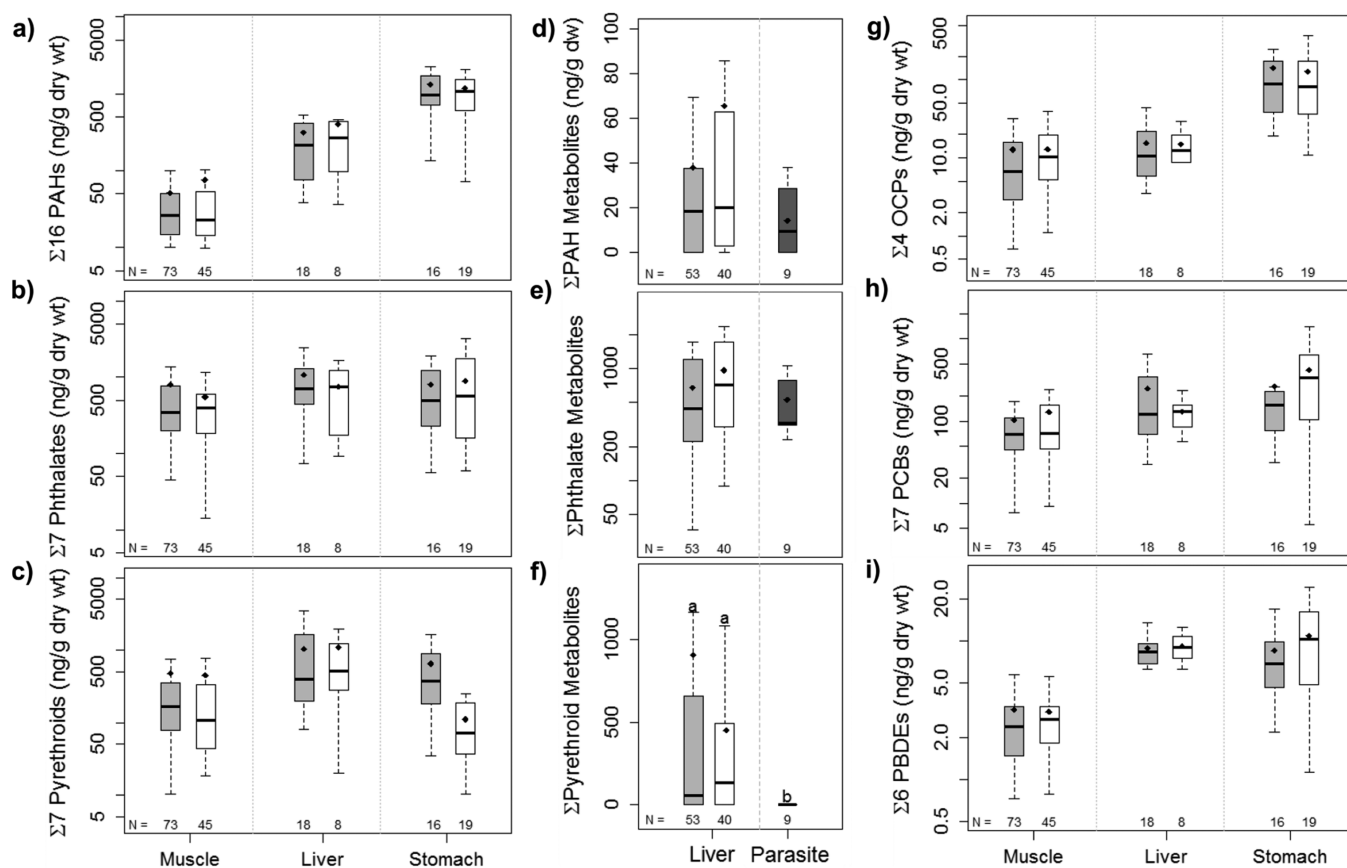


Figure 3. Boxplot of the pollutant load (ng g^{-1} dry wt; [a–c] metabolizable and [d–f] their byproducts, [g–i] persistent) in the muscle, liver, and stomach content of infected (gray) and uninfected (white) chub. Numbers below boxplots refer to the sample size.

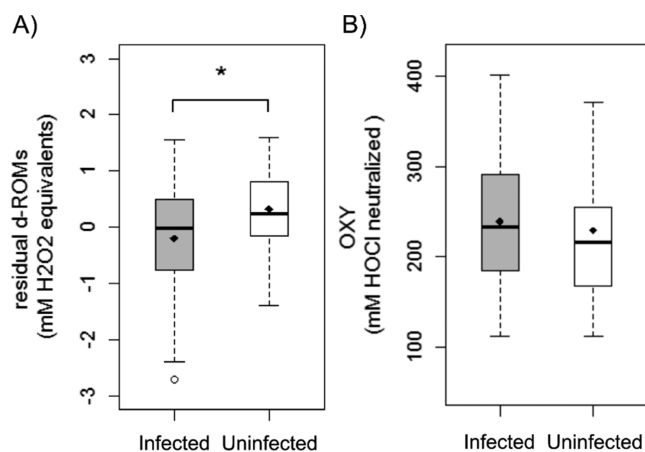


Figure 4. Effect of infection status (parasitized or not) on (A) oxidative damage (d-ROMs) and (B) total plasmatic antioxidant capacity (OXY). Residuals from the final model (see Table S4), excluding the infection status, were used to correct for variation caused by confounding factors (age and $\delta^{15}\text{N}$).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

$F_{(1,109)} = 0.21$, $p = 0.645$; $F_{(1,107.97)} = 0.02$, $p = 0.884$ 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).

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.^{15,16,26,62} Because of their lack of digestive tract and basic synthesis pathways, acanthocephalan parasites take up substances (e.g., lipids) directly from the host intestine.⁶³ 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.⁶⁴ 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.⁶⁵ 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

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.^{23,66} 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^{67,68} 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.⁶⁹

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.¹⁸ 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^{12,14} and persistent pollutants^{68,70} 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,⁷¹ by increasing parasite abundance and diversity in polluted waters⁷² or by compromising the survival of intermediate hosts (e.g., gammarids)⁷³ 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⁻¹ 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 conditions⁴ as enhanced oxidative damage and potentially oxidative stress, in unparasitized chub could drive fitness costs for the host (see the work of Costantini⁷⁴). 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.³⁴ Recent findings also evidenced that parasites were beneficial to crustacean

intermediate hosts exposed to pollution, by increasing antitoxic defenses,^{30,75} 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.^{11,16} Consequently, intestinal parasites may mitigate the production of highly reactive molecules generated from parent pollutants, thereby lowering toxicity.⁵ 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.⁷⁶ 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 Dezfuli et al.⁸ 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,⁴¹ 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.

ACKNOWLEDGMENTS

This work was financially supported by the Seine-Normandy Water Agency (AESN, research program no. 1059502). N.M. was funded by a Ph.D. grant from the Sorbonne University Environmental Transition Institute. The authors are grateful to A. Desportes for her technical assistance, J. O'Rourke and L. Méroth for age determination, M. LeHénaff and S. Papillon for innate immune biomarkers, and M. Mendez and M. Mandeng-Yogo for stable isotope analyses.

REFERENCES

- (1) Canestrari, D.; Bolopo, D.; Turlings, T. C.; Röder, G.; Marcos, J. M.; Baglione, V. From parasitism to mutualism: unexpected interactions between a cuckoo and its host. *Science* **2014**, *343*, 1350–2.
- (2) Redman, R. S.; Dunigan, D. D.; Rodriguez, R. J. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? *New Phytol.* **2001**, *151*, 705–716.
- (3) Shapiro, J. W.; Williams, E. S. C. P.; Turner, P. E. Evolution of parasitism and mutualism between filamentous phage M13 and *Escherichia coli*. *PeerJ* **2016**, *4*, No. e2060.
- (4) Weinersmith, K. L.; Earley, R. L. Better with your parasites? Lessons for behavioural ecology from evolved dependence and conditionally helpful parasites. *Anim. Behav.* **2016**, *118*, 123–133.
- (5) Morrill, A.; Provencher, J. F.; Gilchrist, H. G.; Mallory, M. L.; Forbes, M. R. Anti-parasite treatment results in decreased estimated survival with increasing lead (Pb) levels in the common eider *Somateria mollissima*. *Proc. R. Soc. London, Ser. B* **2019**, *286*, 20191356.
- (6) Pierce, J. J.; Weiner, R. F.; Vesilind, P. A. Water pollution. In *Environmental pollution and control*, 4th ed.; Butterworth-Heinemann, 1998.
- (7) Kennedy, C. R. *Ecology of the Acanthocephala*; Cambridge University Press, New York, NY, 2006.
- (8) Dezfuli, B. S.; Pironi, F.; Giari, L.; Domeneghini, C.; Bosi, G. Effect of *Pomphorhynchus laevis* (Acanthocephala) on putative neuromodulators in the intestine of naturally infected *Salmo trutta*. *Dis. Aquat. Org.* **2002**, *51*, 27–35.
- (9) Bollache, L.; Rigaud, T.; Cézilly, F. Effects of two acanthocephalan parasites on the fecundity and pairing status of female *Gammarus pulex*. *J. Invertebr. Pathol.* **2002**, *79*, 102–110.
- (10) Tain, L.; Perrot-Minnot, M.-J.; Cézilly, F. Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proc. R. Soc. London, Ser. B* **2006**, *273*, 3039–3045.
- (11) Sures, B.; Siddall, R. *Pomphorhynchus laevis*: the intestinal acanthocephalan as a lead sink for its fish host, chub (*Leuciscus cephalus*). *Exp. Parasitol.* **1999**, *93*, 66–72.
- (12) Sures, B.; Siddall, R. *Pomphorhynchus laevis* (Palaecanthocephala) in the intestine of chub (*Leuciscus cephalus*) as an indicator of metal pollution. *Int. J. Parasitol.* **2003**, *33*, 65–70.
- (13) Thielen, F.; Zimmermann, S.; Baska, F.; Taraschewski, H.; Sures, B. The intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) from barbel as a bioindicator for metal pollution in the Danube River near Budapest, Hungary. *Environ. Pollut.* **2004**, *129*, 421–429.
- (14) Filipović Marijić, V.; Vardić Smrzlić, I.; Raspor, B. Effect of acanthocephalan infection on metal, total protein and metallothionein concentrations in European chub from a Sava River section with low metal contamination. *Sci. Total Environ.* **2013**, *463–464*, 772–780.
- (15) Sures, B.; Nachev, M.; Selbach, C.; Marcogliese, D. J. Parasite responses to pollution: What we know and where we go in 'Environmental Parasitology'. *Parasites Vectors* **2017**, *10*, 65.
- (16) Brázová, T.; Hanzelová, V.; Miklisová, D. Bioaccumulation of six PCB indicator congeners in a heavily polluted water reservoir in Eastern Slovakia: tissue-specific distribution in fish and their parasites. *Parasitol. Res.* **2012**, *111*, 779–786.
- (17) Sanchez-Ramirez, C.; Vidal-Martinez, V. M.; Aguirre-Macedo, M. L.; Rodriguez-Canul, R. P.; Gold-Bouchot, G.; Sures, B. *Cichlidogyrus sclerosus* (Monogenea: Ancyrocephalinae) and its host the Nile Tilapia (*Oreochromis niloticus*) as bioindicators of chemical pollution. *J. Parasitol.* **2007**, *93*, 1097–1106.
- (18) Henríquez-Hernández, L. A.; Carretón, E.; Camacho, M.; Montoya-Alonso, J. A.; Boada, L. D.; Valerón, P. F.; Cordón, Y. F.; Almeida-González, M.; Zumbado, M.; Luzardo, O. P. Influence of parasitism in dogs on their serum levels of persistent organochlorine compounds and polycyclic aromatic hydrocarbons. *Sci. Total Environ.* **2016**, *562*, 128–135.
- (19) Malaj, E.; von der Ohe, P. C.; Grote, M.; Kühne, R.; Mondy, C. P.; Usseglio-Polatera, P.; Brack, W.; Schäfer, R. B. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 9549–9554.
- (20) Wang, L. R.; Wang, Y.; Chen, J. W.; Guo, L. H. A structure-based investigation on the binding interaction of hydroxylated polycyclic aromatic hydrocarbons with DNA. *Toxicology* **2009**, *262*, 250–257.
- (21) André, J. M.; Ribeyre, F.; Boudou, A. Mercury contamination levels and distribution in tissues and organs of delphinids (*Stenella attenuata*) from the Eastern Tropical Pacific, in relation to biological and ecological factors. *Mar. Environ. Res.* **1990**, *30*, 43–72.
- (22) Ko, F. C.; Pan, W. L.; Cheng, J. O.; Chen, T. H.; Kuo, F. W.; Kao, S. J.; Chang, C. W.; Ho, H. C.; Wang, W. H.; Fang, L. S. Persistent organic pollutants in Antarctic notothenioid fish and invertebrates associated with trophic levels. *PLoS One* **2018**, *13*, No. e0194147.
- (23) Livingstone, D. R. The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **1998**, *120*, 43–49.
- (24) Yen Le, T.T.; Rijdsdijk, L.; Sures, B.; Jan Hendriks, A. Accumulation of persistent organic pollutants in parasites. *Chemosphere* **2014**, *108*, 145–151.
- (25) Goater, T. M.; Goater, C. P.; Esch, G. W. *Parasitism: The Diversity and Ecology of Animal Parasites*, 2nd ed.; Cambridge University Press, Cambridge, 2014.
- (26) Persson, M. E.; Larsson, P.; Stenroth, P. Biomagnification and polychlorinated biphenyl congener distribution in an aquatic

- predator-prey, host-parasite system. *Environ. Toxicol. Chem.* **2007**, *26*, 837–843.
- (27) Brown, A.; Pascoe, D. Parasitism and host sensitivity to cadmium: an acanthocephalan infection of the freshwater amphipod *Gammarus pulex*. *J. Appl. Ecol.* **1989**, *26*, 473–487.
- (28) Thilakaratne, I. D. S. I. P.; McLaughlin, J. D.; Marcogliese, D. J. Effects of pollution and parasites on biomarkers of fish health in spottail shiners *Notropis hudsonius* (Clinton). *J. Fish Biol.* **2007**, *71*, 519–538.
- (29) Marcogliese, D.; Brambilla, L.; Gagné, F.; Gendron, A. Joint effects of parasitism and pollution on oxidative stress biomarkers in yellow perch *Perca flavescens*. *Dis. Aquat. Org.* **2005**, *63*, 77–84.
- (30) Sánchez, M. I.; Pons, I.; Martínez-Haro, M.; Taggart, M. A.; Lenormand, T.; Green, A. J. When Parasites Are Good for Health: Cestode Parasitism Increases Resistance to Arsenic in Brine Shrimps. *PLoS Pathog.* **2016**, *12*, No. e1005459.
- (31) Marcogliese, D.; Pietrock, M. Combined effects of parasites and contaminants on animal health: parasites do matter. *Trends Parasitol.* **2011**, *27*, 123–130.
- (32) Erkekoglu, P.; Rachidi, W.; Yuzugullu, O. G.; Giray, B.; Favier, A.; Ozturk, M.; Hincal, F. Evaluation of cytotoxicity and oxidative DNA damaging effects of di(2-ethylhexyl)-phthalate (DEHP) and mono(2-ethylhexyl)-phthalate (MEHP) on MA-10 Leydig cells and protection by selenium. *Toxicol. Appl. Pharmacol.* **2010**, *248*, 52–62.
- (33) Frank, S. N.; Faust, S.; Kalbe, M.; Trubiroha, A.; Kloas, W.; Sures, B. Fish hepatic glutathione-S-transferase activity is affected by the cestode parasites *Schistocephalus solidus* and *Ligula intestinalis*: evidence from field and laboratory studies. *Parasitology* **2011**, *138*, 939–944.
- (34) Qu, R.; Feng, M.; Sun, P.; Wang, Z. A comparative study on antioxidant status combined with integrated biomarker response in *Carassius auratus* fish exposed to nine phthalates. *Environ. Toxicol.* **2015**, *30*, 1125–1134.
- (35) Bols, N. C.; Brubacher, J. L.; Ganassin, R. C.; Lee, L. E. J. Ecotoxicology and innate immunity in fish. *Dev. Comp. Immunol.* **2001**, *25*, 853–873.
- (36) Le Guernic, A.; Sanchez, W.; Palluel, O.; Bado-Nilles, A.; Floriani, M.; Turies, C.; Chadili, E.; Della Vedova, C.; Cavalié, I.; Adam-Guillermin, C.; Porcher, J. M.; Geffard, A.; Betoulle, S.; Gagnaire, B. Acclimation capacity of the three-spined stickleback (*Gasterosteus aculeatus*, L.) to a sudden biological stress following a polymetallic exposure. *Ecotoxicology* **2016**, *25*, 1478–1499.
- (37) Fatima, M.; Mandiki, S. N. M.; Douxfils, J.; Silvestre, F.; Coppe, P.; Kestemont, P. Combined effects of herbicides on biomarkers reflecting immune–endocrine interactions in goldfish: Immune and antioxidant effects. *Aquat. Toxicol.* **2007**, *81*, 159–167.
- (38) Kasumyan, A. O. Effects of chemical pollutants on foraging behavior and sensitivity of fish to food stimuli. *J. Ichthyol.* **2001**, *41*, 76–87.
- (39) Santoro, M.; Mattiucci, S.; Work, T.; Cimmaruta, R.; Nardi, V.; Cipriani, P.; Bellisario, B.; Nascetti, G. Parasitic infection by larval helminths in Antarctic fishes: pathological changes and impact on the host body condition index. *Dis. Aquat. Org.* **2013**, *105*, 139–148.
- (40) Hursky, O.; Pietrock, M. Intestinal Nematodes Affect Selenium Bioaccumulation, Oxidative Stress Biomarkers, and Health Parameters in Juvenile Rainbow Trout (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* **2015**, *49*, 2469–2476.
- (41) Maceda-Veiga, A.; Green, A. J.; Poulin, R.; Lagrue, C. Body Condition Peaks at Intermediate Parasite Loads in the Common Bully *Gobiomorphus cotidianus*. *PLoS One* **2016**, *11*, No. e0168992.
- (42) Perrot-Minnot, M. J.; Špakulová, M.; Wattier, R.; Kotlík, P.; Düşen, S.; Aydoğdu, A.; Tougard, C. Contrasting phylogeography of two Western Palaearctic fish parasites despite similar life cycles. *J. Biogeogr.* **2018**, *45*, 101–115.
- (43) Hájková, K.; Pulkrabová, J.; Hajšlová, J.; Randák, T.; Žlábek, V. Chub (*Leuciscus cephalus*) as a bioindicator of contamination of the Vltava River by synthetic musk fragrances. *Arch. Environ. Contam. Toxicol.* **2007**, *53*, 390–396.
- (44) Molbert, N.; Alliot, F.; Santos, R.; Chevreuil, M.; Mouchel, J. M.; Goutte, A. Multi-residue methods for the determination of organic micropollutants and their metabolites in fish tissues. *Environ. Toxicol. Chem.* **2019**, *38*, 1866–1878.
- (45) Penczak, T. Movement pattern and growth ratio of tagged fish in two lowland rivers of central Poland. *Polym. J. Ecol.* **2006**, *54*, 267–282.
- (46) Larno, V.; Laroche, J.; Launey, S.; Flammarion, P.; Devaux, A. Responses of Chub (*Leuciscus cephalus*) Populations to Chemical Stress, Assessed by Genetic Markers, DNA Damage and Cytochrome P4501A Induction. *Ecotoxicology* **2001**, *10*, 145–158.
- (47) Bagenal, T. B. *Fish production in fresh waters*; Blackwell, London, 1978.
- (48) Guardiola, F. A.; Cuesta, A.; Abellán, E.; Meseguer, J.; Esteban, M. A. Comparative analysis of the humoral immunity of skin mucus from several marine teleost fish. *Fish Shellfish Immunol.* **2014**, *40*, 24–31.
- (49) Quade, M. J.; Roth, J. A. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Vet. Immunol. Immunopathol.* **1997**, *58*, 239–248.
- (50) Ellis, A. E. Lysozyme assays. *Technol. fish immunol.* **1990**, *1*, 101–103.
- (51) Costantini, D. Oxidative stress ecology and the d-ROMs test: facts, misfacts and an appraisal of a decade's work. *Behav. Ecol. Sociobiol.* **2016**, *70*, 809–820.
- (52) Beaulieu, M.; Costantini, D. Biomarkers of oxidative status: missing tools in conservation physiology. *Conserv. Physiol.* **2014**, *2*, No. cou014.
- (53) Vaugoyeau, M.; Decencièrre, B.; Perret, S.; Karadas, F.; Meylan, S.; Biard, C. Is oxidative status influenced by dietary carotenoid and physical activity after moult in the great tit (*Parus major*)? *J. Exp. Biol.* **2015**, *218*, 2106–2115.
- (54) Labadie, P.; Alliot, F.; Bourges, C.; Desportes, A.; Chevreuil, M. Determination of polybrominated diphenyl ethers in fish tissues by matrix solid-phase dispersion and gas chromatography coupled to triple quadrupole mass spectrometry: Case study on European eel (*Anguilla anguilla*) from Mediterranean coastal lagoons. *Anal. Chim. Acta* **2010**, *675*, 97–105.
- (55) Hebert, C. E.; Keenleyside, K. A. To normalize or not to normalize? Fat is the question. *Environ. Toxicol. Chem.* **1995**, *14*, 801–807.
- (56) Pinheiro, J.; Bates, D. *Mixed-Effects Models in S and S-PLUS*, 1st ed.; Springer-Verlag: New York, 2000; retrieved from www.springer.com/gp/book/9780387989570.
- (57) R Core Team. *R: A language and environment for statistical computing*; R Foundation for Statistical Computing, Vienna, Austria, 2016.
- (58) Kuznetsova, A.; Brockhoff, P. B.; Christensen, R. H. B. lmerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **2017**, *82*, 82.
- (59) Sures, B.; Siddall, R.; Taraschewski, H. Parasites as Accumulation Indicators of Heavy Metal Pollution. *Parasitol. Today* **1999**, *15*, 16–21.
- (60) US EPA (United States Environmental Protection Agency). *Estimation Programs Interface Suite (EPI Suite) for Microsoft Windows*, v 4.10. Washington, DC, USA, 2012; <http://www.epa.gov/opptintr/exposure/pubs/episuite.htm>.
- (61) Hothorn, T.; Bretz, F.; Westfall, P.; Heiberger, R. M.; Schuetzenmeister, A. *Multcomp. Simultaneous inference in general parametric models*. 2010; <http://cran.r-project.org/web/packages/multcomp/>.
- (62) Oluoch-Otiego, J.; Oyoo-Okoth, E.; Kiptoo, K. K. G.; Chemoiwa, E. J.; Ngugi, C. C.; Simiyu, G.; Omutange, E. S.; Ngure, V.; Opiyo, M. A. PCBs in fish and their cestode parasites in Lake Victoria. *Environ. Monit. Assess.* **2016**, *188*, 483.
- (63) Köhler, P.; Voigt, W. P. Nutrition and metabolism. In *Parasitology in Focus: Facts and Trends*; Mehlhorn, H., Ed.; Springer-Verlag: Berlin, 1988; pp 412–453.

(64) Siddall, R.; Sures, B. Uptake of lead by *Pomphorhynchus laevis* cystacanths in *Gammarus pulex* and immature worms in chub (*Leuciscus cephalus*). *Parasitol. Res.* **1998**, *84*, 573–577.

(65) Vives, I.; Grimalt, J. O.; Ventura, M.; Catalan, J.; Rosseland, B. O. Age dependence of the accumulation of organochlorine pollutants in brown trout (*Salmo trutta*) from a remote high mountain lake (Redó, Pyrenees). *Environ. Pollut.* **2005**, *133*, 343–350.

(66) Ruus, A.; Skaare, J. U.; Ingebrigtsen, K. Accumulation of the lipophilic environmental contaminant lindane in metacercariae of *Bucephaloides gracilescens* (trematoda, bucephalidae) in the central nervous system of bullrout *Myoxocephalus scorpius*. *Dis. Aquat. Org.* **2001**, *48*, 75–77.

(67) Spann, N.; Goedkoop, W.; Traunspurger, W. Phenanthrene Bioaccumulation in the Nematode *Caenorhabditis elegans*. *Environ. Sci. Technol.* **2015**, *49*, 1842–1850.

(68) Henríquez-Hernández, L. A.; Carretón, E.; Camacho, M.; Montoya-Alonso, J. A.; Boada, L. D.; Valerón, P. F.; Falcón-Cordón, Y.; Falcón-Cordón, S.; Almeida-González, M.; Zumbado, M.; Luzardo, O. P. The heartworm (*Dirofilaria immitis*) seems to be able to metabolize organochlorine pesticides and polychlorinated biphenyls: A case–control study in dogs. *Sci. Total Environ.* **2017**, *575*, 1445–1452.

(69) Madenjian, C. P.; Rediske, R. R.; Krabbenhoft, D. P.; Stapanian, M. A.; Chernyak, S. M.; O’Keefe, J. P. Sex differences in contaminant concentrations of fish: a synthesis. *Biol. Sex Differ.* **2016**, *7*, 42.

(70) Vidal-Martínez, V. M.; Aguirre-Macedo, M. L.; Noreña-Barroso, E.; Gold-Bouchot, G.; Caballero-Pinzón, P. I. Potential interactions between metazoan parasites of the Mayan catfish *Ariopsis assimilis* and chemical pollution in Chetumal Bay, Mexico. *J. Helminthol.* **2003**, *77*, 173–184.

(71) Carreras-Aubets, M.; Montero, F. E.; Kostadinova, A.; Carrasson, M. Parasite communities in the red mullet, *Mullus barbatus* L. respond to smallscale variation in the levels of polychlorinated biphenyls in the Western Mediterranean. *Mar. Pollut. Bull.* **2012**, *64*, 1853–1860.

(72) Poulin, R. Toxic pollution and parasitism in freshwater fish. *Parasitol. Today* **1992**, *8*, 58–61.

(73) Gerhardt, A.; Bloor, M.; Mills, C. L. Gammarus: Important Taxon in Freshwater and Marine Changing Environments. *Int. J. Zool.* **2011**, *2011*, 1–2 ID 524276.

(74) Costantini, D. Understanding diversity in oxidative status and oxidative stress: the opportunities and challenges ahead. *J. Exp. Biol.* **2019**, *222*, No. jeb194688.

(75) Gismondi, E.; Cossu-Leguille, C.; Beisel, J. N. Acanthocephalan parasites: Help or burden in gammarid amphipods exposed to cadmium? *Ecotoxicology* **2012**, *21*, 1188–1193.

(76) Li, X. F.; Tian, H. Y.; Zhang, D. D.; Jiang, G. Z.; Liu, W. B. Feeding frequency affects stress, innate immunity and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*. *Fish Shellfish Immunol.* **2014**, *38*, 80–87.

3. Annex 3

Article 3

Molbert N, Angelier A, Alliot F, Ribout C, Goutte A. (*submitted, in revision*) Fish from urban rivers and with high pollutant levels have shorter telomeres. *Biol. Lett.*

Fish from urban rivers and with high pollutant levels have shorter telomeres

Journal:	<i>Biology Letters</i>
Manuscript ID	Draft
Article Type:	Research
Date Submitted by the Author:	n/a
Complete List of Authors:	Molbert, Noëlie; Sorbonne Université, UMR METIS Angelier, Frédéric; CEBC-CNRS UMR 7372, Alliot, Fabrice; EPHE, UMR METIS Ribout, Cécile; CEBC-CNRS UMR 7372, Goutte, Aurélie; EPHE, UMR METIS
Subject:	Ecology < BIOLOGY, Environmental Science < BIOLOGY
Categories:	Physiology
Keywords:	Chub, Telomere, Phthalate, Pesticides, Metabolites

Author-supplied statements

Relevant information will appear here if provided.

Ethics

Does your article include research that required ethical approval or permits?:

Yes

Statement (if applicable):

Fish sampling has been conducted according to 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).

Data

It is a condition of publication that data, code and materials supporting your paper are made publicly available. Does your paper present new data?:

Yes

Statement (if applicable):

Data related to this article are available on Dryad Digital Repository. Dataset, <https://doi.org/10.5061/dryad.bcc2fqz9d>

Reference: Molbert, Noëlie et al. (2020), Fish from urban rivers and with high pollutant levels have shorter telomeres, Dryad, Dataset, <https://doi.org/10.5061/dryad.bcc2fqz9d>

Conflict of interest

I/We declare we have no competing interests

Statement (if applicable):

CUST_STATE_CONFLICT :No data available.

Authors' contributions

This paper has multiple authors and our individual contributions were as below

Statement (if applicable):

A.G. conceived the idea, designed the methodology and acquired the funding. A.G. and F.Alliot contributed to field work. F.Alliot performed chromatographic acquisition. F.Angelier and C.R. performed telomere assays. N.M. performed chemical and data analyses, scales reading and drafted the manuscript with suggestions and comments from F.Angelier, C.R. and A.G. All authors approved the final version of the manuscript.

1 Fish from urban rivers and with high pollutant levels have
2 shorter telomeres

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28 **Abstract:**

29 Environmental pressures, such as urbanization and exposure to pollutants may jeopardize
30 survival of free-living animals. Yet, few studies have investigated such an effect on vertebrate
31 ectotherms, especially regarding the impact of currently-released pollutants. This study aims at
32 testing the effect of urbanization and pollution (phthalate, pesticides: organochlorine [OCP] and
33 pyrethroid pesticides, polychlorobiphenyles: PCB, polybromodiphenylethers: PBDE,
34 polycyclic aromatic hydrocarbons: PAH and some of their metabolites) on telomere length, a
35 suggested biomarker of life expectancy, in the European chub *Squalius cephalus* from urban
36 and rural rivers of the Marne hydrographic network, France. Our results show that telomere
37 length was reduced in chub from urban rivers. Moreover, among the wide range of
38 anthropogenic contaminants investigated, high levels of PCBs in muscle and metabolites of
39 phthalates, PAH and pyrethroids in liver were associated with shorter telomeres in chub. This
40 study suggests that urbanization and chemical pollution may compromise survival of wild fish,
41 by accelerating telomere attrition.

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43 **Keywords:** chub, telomere, metabolites, phthalate, pesticides

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53 **1. Introduction**

54 Chronic exposure to complex mixtures of environmental toxicants may have severe
55 consequences in free-living animals by reducing reproductive outputs and survival [1],
56 thereby leading to population collapse [2]. However, we currently lack robust data to link
57 contaminant burden and survival, probably because studying demographic responses to
58 chemical exposure requires long-term (years to decades) monitoring surveys of numerous
59 marked individuals, which are often difficult to achieve in the wild. Aquatic organisms in
60 urban areas are exposed to a wide array of environmental pollutants, because of sewage and
61 runoff from artificialized surfaces.

62 In that context, telomeres have been recognized as robust molecular tool to predict life
63 expectancy [3,4] and even population vulnerability [5]. Located at the end of eukaryote
64 chromosomes, telomeres shorten through successive cell division. Beyond a critical telomere
65 length, the cell starts to senesce, leading to apoptosis and a decline in tissue function [6].
66 Importantly, this natural process can be accelerated under stressful environmental conditions
67 reviewed in [7,8]. In particular, oxidative stress has been recognized as a mechanistic pathway
68 linking environmental stress and telomere erosion in vertebrates [9,10].

69 Chemical pollution is part of multiple stress factors generating or enhancing oxidative
70 stress [11], yet the effect of chemical exposure on telomere length is poorly known for
71 wildlife, especially for vertebrate ectotherms [12]. To date, studies mostly focused on birds
72 exposed to trace metals [13], chlorinated [14,15] and halogenated compounds [15,16], which
73 are classes of organic chemicals either ban or phase-out from use and production. Currently-
74 release pollutants that are ubiquitous in the environment, such as polycyclic aromatic
75 hydrocarbons (PAHs), phthalate plasticizers and pesticides [17], are known to impair
76 telomere length in human cohorts [18,19]. Although exposure pathways and concentrations
77 might differ, similar effects are expected on aquatic vertebrates. Indeed, vertebrates share the

78 ability to regulate the level of internal organic pollutant exposure *via* metabolic processes [20]
79 that have the potential to produce reactive oxygen species through redox cycling or yield
80 more toxic intermediates than the parent pollutant [21].

81 In addition, many vertebrates are particularly vulnerable to habitat alterations [22],
82 other than chemical pollution. For instance, environmental harshness, disease prevalence and
83 thermal stress have been associated with disturbed oxidative balance and shorter telomeres in
84 fishes [23-27]. In this study, we examined the effect of organic pollutant burden on relative
85 telomere length of the European chub, *Squalius cephalus*, from urban and agricultural
86 habitats. We expected higher chemical pollution on urban rivers [28,29] and predicted that
87 telomeres would be shorter in fish from urban rivers compared to agricultural ones and would
88 decline with pollutant burden.

89

90 **2. Materials and Methods**

91 (a) Samples collection and chemical analyses

92 A total of 118 chub, *S. cephalus*, were caught by electrofishing within twelve days in
93 September 2016 from the Marne River and its tributaries, France, on urban and agricultural
94 areas, representing differently-contaminated riverine habitats (Supplementary material,
95 Appendix A, table S1 and figure S1). Fins were sampled for DNA extraction and scales were
96 removed for age determination (see [30], for details). Muscle (n = 118) and liver (n = 93)
97 samples were stored in polycarbonate tubes to limit phthalate contamination and frozen at -20
98 °C. Metabolites were quantified in fewer individuals since some of them did not yield
99 sufficient biological material to carry out chemical analyses. Analyses of organic pollutants
100 (16 polycyclic aromatic hydrocarbons: PAH; 7 phthalate esters; 7 pyrethroids; 4
101 organochlorine pesticides: OCP; 7 polychlorinated biphenyls: PCB; 6 polybrominated
102 diphenyl ethers: PBDE) and their metabolites (11 hydroxylated PAHs; 9 phthalate

103 monoesters; 4 metabolites of pyrethroids) were performed in muscle and liver respectively,
104 following previously published protocols [31].

105

106 (b) Telomere analysis

107 Telomere length was determined by quantitative PCR (qPCR; BioRad CFX 96, Bio-Rad
108 USA) according [32], adapted for the European chub. Briefly, fin samples were digested with
109 proteinase K and DNA was extracted using the Nucleospin Tissue Kit (Macherey-Nagel),
110 following the manufacturer's instructions. DNA concentration and purity were assessed with
111 a Nanodrop ND1000 spectrophotometer (Thermo Scientific). The telomere primers were
112 similar to those previously used [33]. The control single-copy gene Recombination Activating
113 Gene 1 (RAG-1) was selected and amplified using specific primers [34] designed for the
114 European chub. qPCR was then performed using 2.5 ng of DNA per reaction. The universal
115 telomere primers were respectively used at a concentration of 800 nM, and 300 nM. To
116 generate a six-point standard curve (from 10.0 ng to 0.31 ng) for controlling the amplifying
117 efficiency of the reactions, serial dilutions of DNA from a pooled sampled of 10 chub were
118 included on the plate. All samples were randomly distributed across the PCR plates. The
119 reference sample was run in triplicate to account for inter-plate variation and each sample was
120 run in duplicate on every plate. Intra-plate variation (coefficient of variation: $100 \times SD/\text{mean}$
121 value) for telomere and RAG-1 were 2.07% and 2.80%, respectively. The efficiency of the
122 telomere and RAG-1 assays ranged from 98.8% to 100% and 88.9% to 92.6%, respectively.
123 Overall, 20 samples were excluded due to DNA degradation. As a result, telomere length (T/S
124 ratio) was determined for 98 individuals and was relative to the internal single gene control
125 (RAG-1).

126

127 (c) Statistical analysis

128 To test whether telomere length was affected by organic pollutant burdens, linear mixed
129 model (LMMs) analyses were conducted using the *lme4* and *lmerTest* packages [35,36] in R
130 v. 3.3.2 software [37]. Given that telomere shortened with age in this study ($F_{1,92} = 17.8$, $p \leq$
131 0.001 ; $\beta \pm \text{s.e.} = -0.038 \pm 0.009$) and that telomere attrition is linked to normal aging in fish
132 [38], RTL was first corrected with age (RTLc) to account for different age profiles between
133 individuals. Then, differences in RTLc were assessed with sum-(Σ -) contaminant
134 concentrations (log-transformed) of each pollutant family as fixed effects and habitats as
135 random effects. The significance of random effect was assessed using likelihood ratio tests.
136 We performed diagnostic plots and Shapiro normality tests on residuals to check model
137 assumptions. Details for statistical analyses are presented in the supplementary material
138 (Appendix B).

139 3. Results

140 Relative telomere length (RTL) of chub significantly differ between habitats (t-test: $t = 3.09$, p
141 $= 0.003$) and among sampling sites (ANOVA: $F_{5,92} = 3.73$, $p = 0.004$), even when corrected
142 by age (RTLc; $t = 2.82$, $p = 0.006$, figure 1A). RTL was $\sim 9.82\%$ longer in fish near
143 agricultural areas than those closest to Paris, at proximity to urban habitats. Organic pollutant
144 mean levels \pm standard deviation in chub tissues are listed in table S2 (Supplementary
145 material, Appendix C, table S2). Fish from urban habitats had higher levels of OCPs ($p <$
146 0.001), phthalates ($p = 0.045$) and pyrethroid pesticides ($p = 0.010$) relative to agricultural
147 areas, representing a contamination increase of 48.6%, 20.8% and 15.4%, respectively, but
148 not for PAHs, PBDEs, PCBs and Σ metabolites (all $p \geq 0.126$).

149 Age-corrected telomere length (RTLc) significantly decreased with increasing levels
150 of pollutants, especially for pollutants' by-products (Σ metabolites of phthalates, PAH, and
151 pyrethroids, figure 1B; LMM: $F_{1,71.5} = 6.47$, $p = 0.013$) and PCBs (LMM: $F_{1,91.0} = 5.30$, $p =$
152 0.023 ; Supplementary material, Appendix D, figure S2). Parent pollutants (Σ PAHs,

153 Σ Phthalates, Σ Pyrethroids), Σ OCPs and Σ PBDEs did not show any significant relationship
154 with RTLc (all $F \leq 2.79$, $p \geq 0.097$; Appendix D, table S3).

155

156 **4. Discussion**

157 As previously found in birds [39-42], telomeres were shorter in urban habitats
158 compared to agricultural ones, suggesting higher life-threatening situations for fish in urban
159 rivers. In fact, fish from urban and agricultural rivers did not differ in their pollutant load,
160 except for slightly higher plasticizer and pesticide levels in urban watercourses. Urban river
161 systems have however undergone profound changes, such as damming, banking and
162 channelization that have led to the disruption of longitudinal connectivity, loss of wetlands
163 and spawning grounds, but also increased water temperature, pathogens and boat noise
164 [43,44]. Our study suggest that the diverse and profound degradation of urban streams induce
165 deleterious effects in fish by accelerating telomere attrition and probably jeopardizing their
166 survival. Those results are in line with previous findings, stating that environmental stressors
167 accelerate telomere shortening in avian and fish species [7,24,27].

168 To the best of our knowledge this is the first evidence that exposure to organic
169 pollutants negatively impacts telomere length in fish. In different species of birds, exposure to
170 environmental contaminants (OCPs, perfluoroalkyl substances: PFAS and trace metals) was
171 associated with a general reduction in telomere length [12, 13-15, but see 16]. The originality
172 of this study is to investigate currently-released pollutants and their metabolites in a common
173 freshwater fish species. Among the wide range of analyzed contaminants, the levels of the
174 sum of metabolites (phthalates, PAHs and pyrethroids) were more prone to explain
175 differences in our data than parent pollutants, except for PCBs. In a previous study using the
176 same data set, metabolites of organic pollutants were negatively correlated to antioxidant
177 capacity and peroxidase activity but not to oxidative damage in chub plasma [30]. Even

178 though the underlying mechanisms are still poorly understood, we hypothesize that
179 electrophilic intermediates generated through the metabolization of parent compounds (PAHs,
180 phthalates and pyrethroid pesticides) could increase oxidative attacks by depleting or
181 weakening defence mechanisms (*i.e.*, antioxidants), ultimately shortening telomeres.

182 Our results reveal physiological costs to fish living in polluted urban habitats, which
183 may ultimately jeopardize their survival. Moreover, they highlight the importance of
184 considering metabolites of environmental pollutants to better assess the impacts of currently-
185 released chemicals on wildlife.

186

187 **Ethics**

188 Fish sampling has been conducted according to relevant national and European guidelines
189 (L436-9, EN14011). The authorization for the scientific fish capture was delivered by local
190 administration authorities (Departmental Direction of Territories of Seine-et-Marne).

191

192 **Data accessibility**

193 Data related to this article are available on Dryad Digital Repository. Dataset,
194 <https://doi.org/10.5061/dryad.bcc2fqz9d>

195

196 **Authors' contributions**

197 A.G. conceived the idea, designed the methodology and acquired the funding. A.G. and
198 F.Alliot contributed to field work. F.Alliot performed chromatographic acquisition.
199 F.Angelier and C.R. performed telomere assays. N.M. performed chemical and data analyses,
200 scales reading and drafted the manuscript with suggestions and comments from F.Angelier,
201 C.R. and A.G. All authors approved the final version of the manuscript.

202

203 **Funding**

204 PhD funding was provided by the Sorbonne University Environmental Transition Institute
205 (SU-ITE). The work described was financially supported by the Seine-Normandy Water
206 Agency (AESN, research program n° 1059502), and by the CPER ECONAT.

207

208 **Acknowledgements**

209 The authors are very grateful to Annie Desportes for her technical assistance.

210

211 **Supplementary material**

212 Appendix A – Habitat determination, Appendix B – Statistical Methods and Appendix C –
213 Descriptive statistics and Appendix D – Model outputs

214

215 **References**

- 216 [1] Goutte A, Barbraud C, Meillère A, Carravieri A, Bustamante P, Labadie P, Budzinski H,
217 Delord K, Cherel Y, Weimerckirch H, Chastel O. 2014 Demographic consequences of
218 heavy metals and persistent organic pollutants in a vulnerable long-lived bird, the
219 wandering albatross. *Proc. R. Soc. B* **281**, 20133313. (doi:10.1098/rspb.2013.3313)
- 220 [2] Desforges JP, Hall A, McConnell B, Rosing-Asvid A, Barber JL, Brownlow A, De Guise
221 S, Eulaers I, Jepson PD, Letcher RJ, Levin M, Ross PS, Samarra F, Víkingsson G,
222 Sonne C, Dietz D. 2018 Predicting global killer whale population collapse from PCB
223 pollution. *Science* **361**, 1373–1376. (doi:10.1126/science.aat1953)
- 224 [3] Tricola GM, Simons MJP, Atema E, Boughton RK, Brown JL, Dearborn DC, Divoky G,
225 Eimes JA, Huntington CE, Kitaysky AS, Juola FA, Lank DB, Litwa HP, Mulder EGA,
226 Nisbet ICT, Okanoya K, Safran RJ, Schoech SJ, Schreiber EA, Thompson PM,
227 Verhulst S, Wheelwright NT, Winkler DW, Young RC, Vleck CM, Hausmann MF.
228 2018 The rate of telomere loss is related to maximum lifespan in birds. *Phil. Trans. R.*
229 *Soc. B* **373**, 20160445. (doi:10.1098/rstb.2016.0445)
- 230 [4] Whittemore K, Vera E, Martínez-Nevado E, Sanpera C, Blasco MA. 2019 Telomere
231 shortening rate predicts species life span. *P. Natl. Acad. Sci. USA* **116**, 15122–15127.
232 (doi:10.1073/pnas.1902452116)

- 233 [5] Dupoué A, Rutschmann A, Le Galliard JF, Clobert J, Angelier F, Marciau C, Ruault S,
234 Miles D, Meylan S. 2017 Shorter telomeres precede population extinction in wild
235 lizards. *Sci. Rep.* **7**, 16976. (doi:10.1038/s41598-017-17323-z)
- 236 [6] Monaghan P, Haussmann M. 2006 Do telomere dynamics link lifestyle and lifespan?
237 *Trends Ecol. Evol.* **21**, 47–53. (doi:10.1016/j.tree.2005.11.007)
- 238 [7] Angelier F, Costantini D, Blévin P, Chastel O. 2018 Do glucocorticoids mediate the link
239 between environmental conditions and telomere dynamics in wild vertebrates? A
240 review. *Gen Comp Endocrinol.* **256**, 99–111. (doi:10.1016/j.ygcen.2017.07.007)
- 241 [8] Chatelain M, Drobniak SM, Szulkin M. 2020 The association between stressors and
242 telomeres in non-human vertebrates: a meta-analysis. *Ecol Lett.* **23**, 381–398.
243 (doi:10.1111/ele.13426)
- 244 [9] von Zglinicki T. 2002 Oxidative stress shortens telomeres. *Trends Biochem. Sci.* **27**, 339–
245 344. (doi:10.1016/S0968-0004(02)02110-2)
- 246 [10] Riechert S, Stier A. 2017 Does oxidative stress shorten telomeres *in vivo*? A review. *Biol*
247 *Lett.* **13**, 20170463. (doi:10.1098/rsbl.2017.0463)
- 248 [11] Yazdani M. 2018 Comparative toxicity of selected PAHs in rainbow trout hepatocytes:
249 genotoxicity, oxidative stress and cytotoxicity. *Drug Chem. Toxicol.* **43**, 1–8.
250 (doi:10.1080/01480545.2018.1497054)
- 251 [12] Louzon M, Coeurdassier M, Gimbert F, Pauget B, de Vaufleury A. 2019 Telomere
252 dynamic in humans and animals: Review and perspectives in environmental
253 toxicology. *Environ Int.* **131**, 105025. (doi:10.1016/j.envint.2019.105025)
- 254 [13] Stauffer J, Panda B, Eeva T, Rainio M, Ilmonen P. 2017 Telomere damage and redox
255 status alterations in free-living passerines exposed to metals. *Sci. Total Environ.* **575**,
256 841e848. (doi:10.1016/j.scitotenv.2016.09.131)
- 257 [14] Blévin P, Angelier F, Tartu S, Ruault S, Bustamante P, Herzke D, Moe B, Bech C,
258 Gabrielsen GW, Bustnes JO, Chastel O, 2016 Exposure to oxychlordan is associated
259 with shorter telomeres in arctic breeding kittiwakes. *Sci. Total Environ.* **563**, 125e130.
260 (doi:10.1016/j.scitotenv.2016.04.096)
- 261 [15] Sletten S, Bourgeon S, Bårdsen BJ, Herzke D, Criscuolo F, Massemin S, Zahn S,
262 Johnsen TV, Bustnes JO. 2016 Organohalogenated contaminants in whitetailed eagle
263 (*Haliaeetus albicilla*) nestlings: an assessment of relationships to immunoglobulin
264 levels, telomeres and oxidative stress. *Sci. Total Environ.* **539**, 337e349.
265 (doi:10.1016/j.scitotenv.2015.08.123)

- 266 [16] Blévin P, Angelier F, Tartu S, Bustamante P, Herzke D, Moe B, Bech C, Wing
267 Gabrielsen G, Bustnes JO, Chastel O. 2017 Perfluorinated substances and telomeres in
268 an Arctic seabird: Cross-sectional and longitudinal approaches. *Environ. Poll.* **230**,
269 360–367. (doi:10.1016/j.envpol.2017.06.060)
- 270 [17] Malaj E, von der Ohe PC, Grote M, Kühne R, Mondy CP, Usseglio-Polatera P, et al.
271 2014 Organic chemicals jeopardize the health of freshwater ecosystems on the
272 continental scale. *P. Natl. Acad. Sci. USA* **111**, 9549–9554.
273 (doi:10.1073/pnas.1321082111)
- 274 [18] Hoxha M, Dioni L, Bonzini M, Pesatori AC, Fustinoni S, Cavallo D, Carugno M, Albetti
275 B, Marinelli B, Schwartz J, Bertazzi PA, Baccarelli A. 2009 Association between
276 leukocyte telomere shortening and exposure to traffic pollution: a cross-sectional study
277 on traffic officers and indoor office workers. *Environ. Health* **8**, 41.
278 (doi:10.1186/1476-069X-8-41)
- 279 [19] Hou L, Andreotti G, Baccarelli AA, Savage S, Hoppin JA, Sandler DP, Barker J, Zhu
280 ZZ, Hoxha M, Dioni L, Zhang X, Koutros S, Freeman LE, Alavanja MC. 2013
281 Lifetime pesticide use and telomere shortening among male pesticide applicators in
282 the Agricultural Health Study. *Environ. Health perspect.* **121**, 919–924. (doi:
283 10.1289/ehp.120643)
- 284 [20] Barron MG, Albro PW, Hayton WL. 1995 Biotransformation of di(2-
285 ethylhexyl)phthalate by rainbow trout. *Environ. Toxicol. Chem.* **14**, 873–876.
286 (doi:10.1002/etc.5620140519)
- 287 [21] Wang LR, Wang Y, Chen JW, Guo LHA. 2009 A structure-based investigation on the
288 binding interaction of hydroxylated polycyclic aromatic hydrocarbons with DNA.
289 *Toxicology* **262**, 250–257. (doi:10.1016/j.tox.2009.06.015)
- 290 [22] Ripple WJ, Wolf C, Newsome TM, Hoffmann M, Wirsing AJ, McCauley DJ. 2017
291 Extinction risk is most acute for the world's largest and smallest vertebrates. *Proc Natl*
292 *Acad Sci U S A.* **114**, 10678–10683. (doi:10.1073/pnas.1702078114)
- 293 [23] Rollings N, Miller E, Olsson M. 2014 Telomeric attrition with age and temperature in
294 Eastern mosquitofish (*Gambusia holbrooki*). *Naturwissenschaften* **101**, 241–244.
295 (doi:10.1007/s00114-014-1142-x)
- 296 [24] Jasinska EJ, Goss GG, Gillis PL, Van Der Kraak GJ, Matsumoto J, de Souza Machado
297 AA, Giacomini M, Moon TW, Massarsky A, Gagné F, Servos MR, Wilson J, Sultana
298 T, Metcalfe CD. 2014 Assessment of biomarkers for contaminants of emerging

- 299 concern on aquatic organisms downstream of a municipal wastewater discharge. *Sci*
300 *Total Environ.* **530–531**, 140–153. (doi:10.1016/j.scitotenv.2015.05.080)
- 301 [25] Debes PV, Visse M, Panda B, Ilmonen P, Vasemägi A. 2016 Is telomere length a
302 molecular marker of past thermal stress in wild fish? *Mol. Ecol.* **25**, 5412–5424.
303 (doi:10.1111/mec.13856)
- 304 [26] McLennan D, Armstrong JD, Stewart DC, Mckelvey S, Boner W, Monaghan P, Metcalfe
305 NB. 2016 Interactions between parental traits, environmental harshness and growth
306 rate in determining telomere length in wild juvenile salmon. *Mol. Ecol.* **25**, 5425–
307 5438. (doi: 10.1111/mec.13857)
- 308 [27] Stauffer J, Bruneaux M, Panda B, Visse M, Vasemägi A, Ilmonen P. 2017 Telomere
309 length and antioxidant defense associate with parasite-induced retarded growth in wild
310 brown trout. *Oecologia* **185**, 365–374. (doi:10.1007/s00442-017-3953-x)
- 311 [28] Teil MJ, Blanchard M, Moreau-Guigon E, Dagnat C, Alliot F, Bourges C, Desportes A,
312 Chevreuil M. 2013 Phthalate Fate in the Hydrographic Network of the River Seine
313 Basin (France) Under Contrasted Hydrological Conditions. *Water Air Soil Poll.* **224**,
314 1592. (doi:10.1007/s11270-013-1592-3)
- 315 [29] Peng C, Wang M, Chen W. 2016 Spatial Analysis of PAHs in Soils along an Urban–
316 Suburban–Rural Gradient: scale effect, distribution patterns, diffusion and influencing
317 factors. *Sci. Rep.* **6**, 37185. (doi:10.1038/srep37185)
- 318 [30] Molbert N, Alliot A, Leroux-Coyau M, Médoc V, Biard C, Meylan S, Jacquin L, Santos
319 R, Goutte A. (in press) Potential benefits of acanthocephalan parasites for chub hosts
320 in polluted environments. *Environ. Sci. Technol.*
- 321 [31] Molbert N, Alliot F, Santos R, Chevreuil M, Mouchel JM, Goutte A. 2019 Multi-residue
322 methods for the determination of organic micropollutants and their metabolites in fish
323 tissues. *Environ. Toxicol. Chem.* **38**, 1866–1878. (doi:10.1002/etc.4500)
- 324 [32] Dupoué A, Angelier F, Ribout C, Meylan S, Rozen-Rechels D, Decencièrre B, Agostini
325 S, Le Galliard JF. 2020 Chronic water restriction triggers sex-specific oxidative stress
326 and telomere shortening in lizards. *Biol. Lett.* **16**, 20190889.
327 (doi:10.1098/rsbl.2019.0889).
- 328 [33] Petitjean Q, Jean S, Côte J, Larcher T, Angelier F, Ribout C, Perrault A, Laffaille P,
329 Jacquin L. 2020 Direct and indirect effects of multiple environmental stressors on fish
330 health in human-altered rivers. *Sci. Total Environ.* 140657.
331 (doi:10.1016/j.scitotenv.2020.140657)

- 332 [34] McLennan D, Recknagel H, Elmer KR, Monaghan P. 2019 Distinct telomere differences
333 within a reproductively bimodal common lizard population. *Funct. Ecol.* **33**, 1917–
334 1927. (doi:10.1111/1365-2435.13408)
- 335 [35] Pinheiro J, Bates D. 2000 Mixed-Effects Models in S and S-PLUS (1st ed). New York:
336 Springer-Verlag. (Retrieved from //www.springer.com/gp/book/9780387989570)
- 337 [36] Kuznetsova A, Brockhoff PB, Christensen RHB. 2017 lmerTest Package: Tests in Linear
338 Mixed Effects Models. *J. Stat. Softw.* **82**. (doi:10.18637/jss.v082.i13)
- 339 [37] R Core Team. 2016 R: A language and environment for statistical computing. R
340 Foundation for Statistical Computing, Vienna, Austria.
- 341 [38] Hatakeyama H, Yamazaki H, Nakamura KI, Izumiya-Shimomura N, Aida J, Suzuki
342 H, Tsuchida S, Matsuura M, Takubo K, Ishikawa N. 2016 Telomere attrition and
343 restoration in the normal teleost *Oryzias latipes* are linked to growth rate and
344 telomerase activity at each life stage. *Aging* **8**, 62–75. (doi:10.18632/aging.100873)
- 345 [39] Meillere A, Brischoux F, Ribout C, Angelier F. 2015 Traffic noise exposure affects
346 telomere length in nestling house sparrows. *Biol. Lett.* **11**, 1–5.
347 (doi:10.1098/rsbl.2015.0559)
- 348 [40] Salmón P, Nilsson JF, Nord A, Bensch S, Isaksson C. 2016 Urban environment shortens
349 telomere length in nestling great tits, *Parus major*. *Biol. Lett.* **12**, 20160155.
350 (doi:10.1098/rsbl.2016.0155)
- 351 [41] Ibáñez-Álamo JD, Pineda-Pampliega J, Thomson RL, Aguirre JI, Díez-Fernández A,
352 Faivre B, Verhulst S. 2018 Urban blackbirds have shorter telomeres. *Biol. Lett.* **14**,
353 20180083. (doi:10.1098/rsbl.2018.0083)
- 354 [42] Grunst AS, Grunst ML, Bervoets L, Pinxten R, Eens M. 2020 Proximity to roads, but not
355 exposure to metal pollution, is associated with accelerated developmental telomere
356 shortening in nestling great tits. *Environ. Poll.* **256**, 113373.
- 357 [43] Paul MJ, Meyer JL. 2001 Streams in the urban landscape. *Annu. Rev. Ecol. Syst.* **32**,
358 333–365. (doi:10.1146/annurev.ecolsys.32.081501.114040)
- 359 [44] Hanache P, Spataro T, Firmat C, Boyer N, Fonseca P, Médoc V. 2020 Noise-induced
360 reduction in the attack rate of a planktivorous freshwater fish revealed by functional
361 response analysis. *Freshwater Biol.* **65**, 75–85. (doi:10.1111/fwb.13271)
- 362
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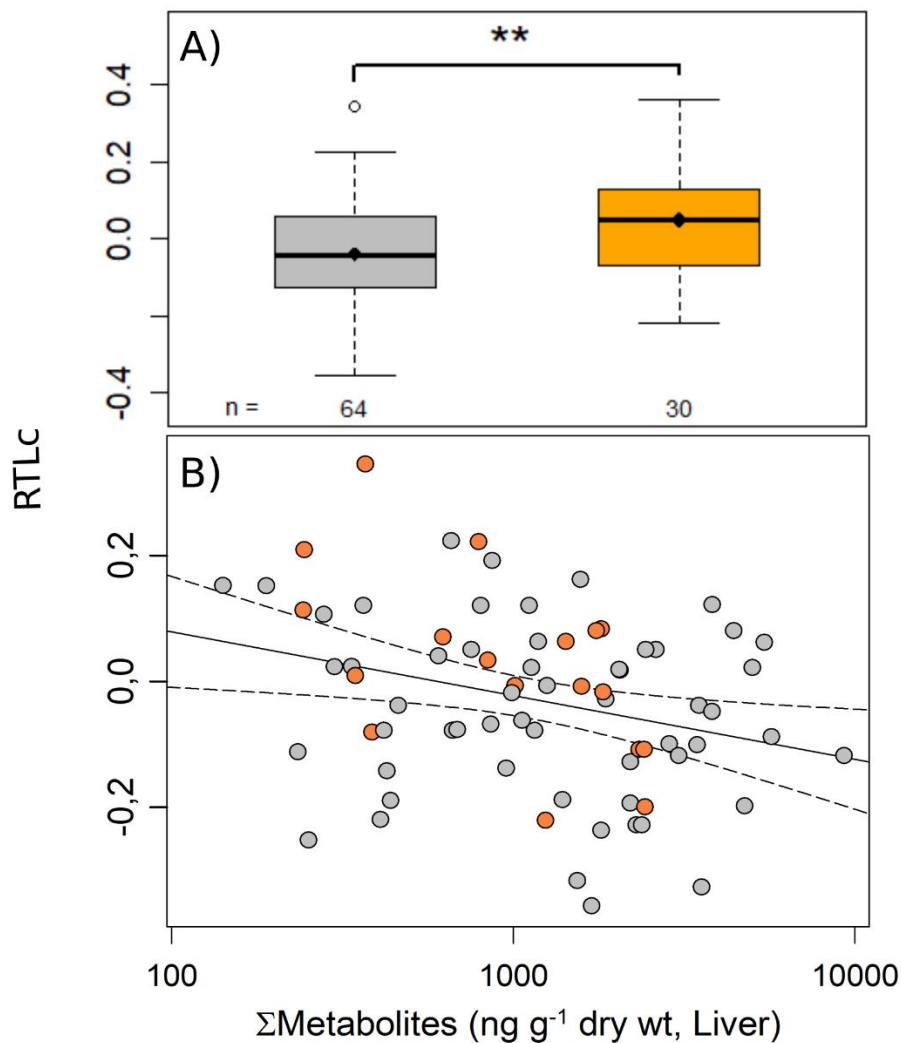
364 **FIGURE CAPTIONS**

365

366 **FIGURE 1.** Age-corrected telomere length (RTLc, residuals $RTL \sim \text{age}$) of chub depending on
367 A) each habitat (urban: gray and agricultural: orange) and B) on the levels of metabolites ⁽¹⁾
368 (Σ Metabolites, ng g^{-1} dry wt, liver). Filled diamonds represent the arithmetic mean and **
369 indicates a significant difference ($p < 0.001$). Numbers represent sample size. Dashed lines
370 represent the 95% confidence interval

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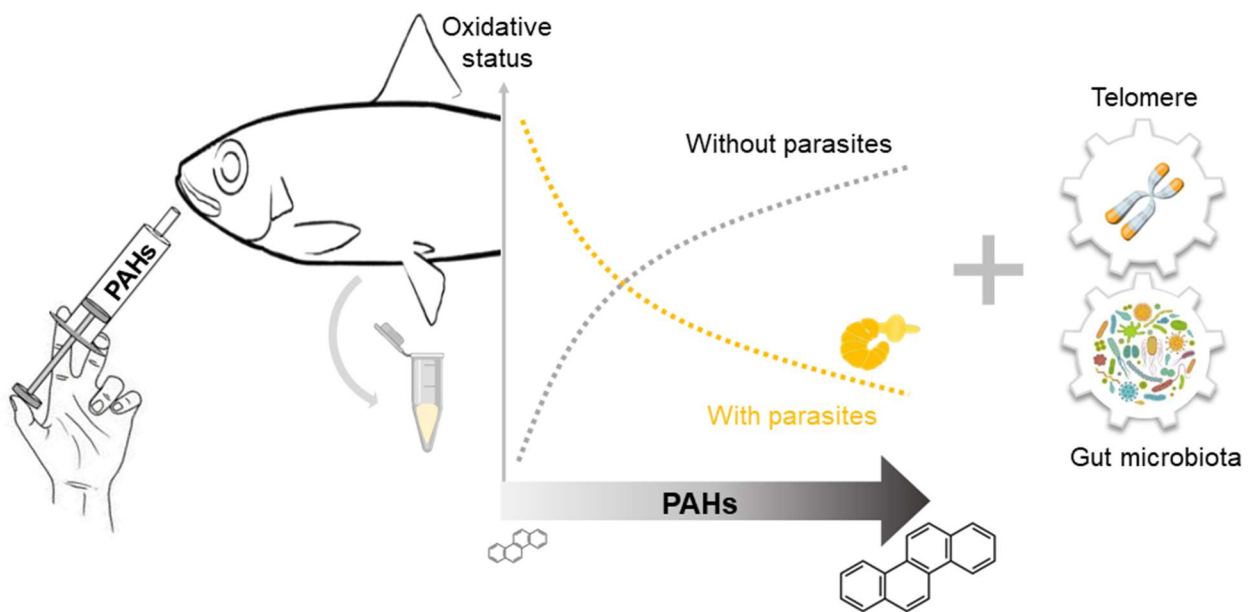
FIGURE 1.



⁽¹⁾ Σ Metabolites: mono-methyl phthalate, mono-ethyl phthalate, mono-iso-butyl phthalate, mono-*n*-butyl phthalate, mono-benzyl phthalate, mono-*n*-octyl phthalate, mono-2-ethylhexyl phthalate, mono-2-ethyl-5-oxohexyl phthalate, mono-2-ethyl-5-hydroxyhexyl phthalate, 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 3-hydroxyfluorene, 9-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, 1-hydroxypyrene, 6-hydroxychrysene, 3-hydroxybenzo(a)pyrene, 3-(2,2-dichlorovinyl)-2,2-dimethyl-1-cyclopropane: *cis*-DCCA and *trans*-DCCA, 4-fluoro-3-phenoxybenzoic acid, 3-phenoxybenzoic acid

Article 4

Molbert N, Colin Y, Agostini S, Alliot F, Angelier F, Berthe T, Biard C, Decencière B, Leroux-Coyau M, millot³, Cécile Ribout, Fabienne Petit and Aurélie Goutte. (*Submitted*) Parasitism reduces oxidative stress and alters gut microbiota of fish host experimentally exposed to PAHs.



1 Parasitism reduces oxidative stress and alters gut microbiota
2 of fish host experimentally exposed to PAHs

3
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26
27 **Keywords:** Parasite, gut microbiota, metabolites, oxidative status, telomeres, PAHs

33 **Summary Statement**

34 Intestinal parasites can modulate the host's stress response to toxicants through physiological
35 changes, which might benefit the host under polluted condition.

36

37 **ABSTRACT**

38 Some parasites may interfere with the fate of environmental pollutants within their host
39 through their capacity to bioaccumulate them. However, the question remains whether this
40 physiological trait may benefit their hosts in polluted environments. We experimentally tested
41 the effect of chemical exposure on the European chub (*Squalius cephalus*) naturally infected
42 or uninfected with acanthocephalan parasites, by monitoring condition indices, the oxidative
43 status, telomere length and composition of gut bacterial communities. Overall, five weeks
44 exposure to polycyclic aromatic hydrocarbons (PAHs) at three levels (0.1X, 1X, 10X
45 environmental exposure) did not compromise survival nor body condition of infected hosts.
46 Although parasite infection did not significantly reduce the levels of PAHs in host tissues, nor
47 those of liver metabolites, infection had an impact on the oxidative status. While uninfected
48 fish showed lower oxidative damage than parasitized ones at 0.1X, this effect was reversed at
49 higher exposure. Meanwhile, antioxidant capacity did not differ in response to parasite
50 infection nor PAHs exposure. Similarly, parasite infection and PAHs exposure had no
51 deleterious effect on telomere length of the fish host. Interestingly, fish gut microbiota
52 diversity differed after PAHs exposure as compared to fish not experimentally exposed to
53 PAHs (T₀), and among infected hosts as compared to uninfected ones, with a marked increase
54 in relative abundances of *Tenericutes* and *Fusobacteria*. Our results suggest that intestinal
55 parasites may change the composition of gut microbiota. This experimental study provides the
56 first evidence that the outcome of host-parasite interactions can shift from negative to positive
57 as pollutant exposure increases.

58

59 **INTRODUCTION**

60 Shifts from exploitative behaviours (*i.e.*, parasitism) to mutualistic interactions may naturally
61 occur under stressful biotic and environmental conditions, in a variety of terrestrial and
62 aquatic ecosystems (Redman et al., 2001; Gismondi et al., 2012 ; Canestrari et al., 2014;
63 Sánchez et al., 2016; Shapiro et al., 2016; Morrill et al., 2019). Recently, we investigated how
64 the presence of acanthocephalan parasites *Pomphorhynchus* sp. affected the physiology of its
65 freshwater fish host, the European chub *Squalius cephalus*, in polluted environments (Molbert

66 et al., 2020). These parasites are endoparasitic worms that are trophically transmitted from an
67 intermediate crustacean host to the intestine of a vertebrate definitive host (Kennedy, 2006).
68 Their establishment in the final host is usually associated with mechanical damage and
69 inflammation of the intestinal wall (Taraschewski, 2000), sometimes reducing the growth and
70 survival of infected organisms (Sakthivel et al., 2016; Silva-Gomes et al., 2017). From field
71 studies, however, we evidenced significantly lower oxidative damage in parasitized fish
72 inhabiting chemically-altered environments (Molbert et al., 2020). Likely, the capacity of
73 acanthocephalan parasites to accumulate pollutants (organic pollutants: polychlorinated
74 biphenyls: PCBs and polycyclic aromatic hydrocarbons: PAHs, Brázová et al., 2012; Oluoch-
75 Otiego et al., 2016; Akinsanya et al., 2020, trace metal elements: Sures and Siddall, 1999;
76 Sures and Siddall, 2003; Thielen et al., 2004; Filipović Marijić et al., 2013) may lead to the
77 detoxification of the infected organisms, thereby changing the distribution and toxicity of
78 chemicals in the host. Accordingly, *Pomphorhynchus* sp., was considered beneficial for its
79 host under stressful chemical conditions (Weinersmith et al., 2016). In order to disentangle
80 potential confounding factors that are inherent to correlative field studies, the present study
81 aims to experimentally test the benefits of parasites in wild fish exposed to pollutants.

82 PAHs are recognized as a main cause of deterioration of aquatic ecosystems,
83 especially in Europe (Malaj et al., 2014; European Environment Agency, 2018), as a
84 consequence of human activities. Mean levels of PAHs in acanthocephalans can reach up to
85 700 times that in the host (Molbert et al., 2020), suggesting a bioaccumulation in parasite
86 tissues. Additionally, PAHs are readily biotransformed and excreted by vertebrates through
87 bile and urine (De Maagd and Vethaak, 1998). However, their biotransformation by liver
88 enzymes into water-soluble derivatives may generate reactive electrophilic intermediates,
89 which are sometimes more noxious than their corresponding parent molecules (Wang et al.,
90 2009). As a result of their rapid metabolism, analyses restricted to parent compounds might
91 underestimate PAH exposure and obscure potential links to biomarker responses. So far, fish
92 exposure to PAHs has been associated with a wide range of biological dysfunctions, such as
93 immunosuppression, decreased body condition, as well as adverse developmental and
94 reproductive effects (Reynaud and Deschaux, 2006; Snyder et al., 2019). In addition, PAHs
95 may affect oxidative status in fish species (Livingstone, 2001; Santana et al., 2018). First,
96 biotransformation of PAHs may generate reactive oxygen species (ROS) through redox
97 cycling. Second, increasing levels of PAH metabolites have been associated with decreasing
98 peroxidase activity, a ROS scavenger (Molbert et al., 2020). When the production of pro-
99 oxidant compounds exceeds that of their neutralization by antioxidant defenses, oxidative

100 stress arises, leading to potential damage to molecular and cellular components (Soltani et al.,
101 2019). Still, it remains challenging to ascertain the relationship between the exposure to one
102 class of pollutant and its effects on biota, especially given the multiple factors influencing the
103 fate of pollutants inside organisms.

104 Recent studies have evidenced the role of the gut bacterial community (*i.e.*,
105 microbiome) in resistance towards environmental pollutants. The metabolic capacity of gut
106 microbiota being similar to that of the liver (Li and Jia, 2013), bacterial communities play key
107 roles in the bioavailability and metabolism of various xenobiotics (Gaulke et al, 2016),
108 including PAHs (Claus et al., 2016). Perceived as a valuable physiological marker of stress,
109 the microbiota has been increasingly studied in ecotoxicological studies (Evariste et al.,
110 2019), being essential to draw up a health assessment of individuals. According to previous
111 studies, changing environmental conditions (contaminants) and ecological interactions
112 (parasitism) shape the composition of gut microbiota, as well as their subsequent role in host
113 physiology and immunity (reviewed in Butt and Volkoff, 2019). By inhibiting bacterial
114 growth or inducing dysbiosis (*i.e.*, microbiome disruptions), environmental pollutants can
115 affect the metabolic activity of gut microbiome, thereby impacting their toxicity and
116 bioavailability in the body (Gaulke et al., 2016; Bagi et al., 2018; Zhao et al., 2019).
117 Additionally, vertebrates' gut microbiota can be altered by the hosts' parasites. Intestinal
118 parasites, such as acanthocephalan, share the same ecological niche than microbial
119 communities, and both are therefore capable of interacting with each other (Newbold et al.,
120 2016, Fu et al., 2019, Ling et al., 2020). Both stressors, parasites and pollutants, are then
121 expected to have interactive effects on the composition and/or diversity of gut microbiota,
122 with potential fitness consequences.

123 Through their adverse effects, environmental stressors (parasite infection and chemical
124 exposure) may ultimately lead to fitness consequences, such as reduced animal's survival.
125 Recently, telomeres have been recognized as robust molecular tool to predict life expectancy
126 (Angelier et al., 2019; Whittemore et al., 2019). Located at the end of eukaryotic
127 chromosomes, telomeres shorten through successive cell division and under the exposure of
128 oxidative stress (Reichert and Stier, 2017). Independently, both parasite infection (Asghar et
129 al., 2015; Karell et al., 2017) and chemical pollution (Blévin et al., 2016; Sletten et al., 2016;
130 Stauffer et al., 2017; Molbert et al., *under review*) have been previously associated with
131 shorter telomeres in wild organisms. While the underlying mechanisms remain still unclear,
132 telomere length could provide valuable information on the potential fitness outcome of
133 parasite infection in contaminated environments.

134 To address the effect of chemical pollution on host-parasite interaction, a cross
135 experimental design was performed on wild European chubs, naturally infected or non-
136 infected by *Pomphorhynchus* sp., exposed to environmentally relevant PAH levels. By
137 examining general biomarkers, which respond to parasite infection and pollutant exposure, we
138 investigated the health status of the fish host, and in particular, oxidative stress, telomere
139 length and the diversity and composition gut microbiome vary in accordance with both stress
140 factors. We predicted that: (1) infected hosts would have lower concentrations of PAHs in
141 their tissues than uninfected ones due to the bioaccumulation process within parasites, (2)
142 exposure to PAHs would increase reactive oxygen metabolite (ROM) concentrations and
143 decrease antioxidant capacity in fish plasma, thereby shortening telomere length, (3) this
144 effect would be reduced in infected chubs given the capacity of parasites to accumulate
145 toxicants; and (4) exposure to PAHs would impact bacterial diversity of the host gut
146 microbiota, with stronger effects for uninfected chubs compared to parasitized ones.

147

148 MATERIAL AND METHODS

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

154

155 Capture and housing conditions

156 One population of European chub, *S. cephalus*, was selected on a tributary of the Marne River
157 (49°5'42''N, 3°40'23''E) where the presence of intestinal parasites *Pomphorhynchus* sp. has
158 been previously recorded (65 %) and the levels of contamination is known (see Molbert et al.,
159 2020). To control for variables susceptible to affect biomarker responses, chub were selected
160 based on specific body length ($n = 160$; mean \pm SD; 56.7 ± 28.2 g; 16.6 ± 2.67 cm),
161 corresponding to immature individuals, electrofished within one week in January 2019. Fish
162 were returned rapidly in 100-L basins filled with well-aerated river water to the CEREEP-
163 Ecotron facilities and maintained for one week in outdoor tanks ($n = 5, 3 \text{ m}^3$) under natural
164 conditions of temperature. Anesthetized chub ($M222, 80 \text{ mg L}^{-1}$) were then carefully tagged
165 with a passive integrated transponder device (8mm x 1.4mm FDX-B skinny tag, OREGON
166 RFID Portland, USA) inserted intraperitoneally, weighted (± 0.5 g) and measured (± 0.1 cm).
167 Tagged fish were randomly divided into sixteen 175-L tanks (80 x 60 x 42 cm) and were

168 acclimated for 2 weeks before being exposed to PAHs. Chub being gregarious, a density of
169 10 fish per tank was chosen to limit stressful conditions. Tanks consisted of continuously
170 aerated systems, equipped with oxygen pumps and mechanical filters, distributed randomly
171 within the experimental room and under a photoperiod regime fixed at a 12:12 h light/dark
172 cycle. Water temperature was progressively warmed up to indoor conditions during the
173 acclimation period and kept constant during the experimental activity ($11.6 \pm 0.51^\circ\text{C}$). Fish
174 were fed twice daily with commercial fish pellets (Tetra). Half of the water was renewed
175 every 2 days and physico-chemical parameters were monitored and held constant during the
176 experiment (mean \pm SD: pH 7.93 ± 0.09 , $187 \pm 5.21 \mu\text{S cm}^{-1}$, O_2 $9.60 \pm 0.29 \text{ mg L}^{-1}$,
177 saturation $88.4 \pm 2.98\%$).

178

179 Experimental design

180 A subsample of ten fish were sacrificed before the first contamination (T_0) to establish basal
181 levels of PAHs in chub, assess their oxidative status and composition of gut microbiota (Fig.
182 1). Five-week experimental PAH exposure was then performed on three groups of fifty
183 naturally unparasitized and infected fish (determined by dissection). Treatment groups were
184 assigned randomly among fifteen exposure tanks, five tanks per group, to allow the evaluation
185 of the effects of parasite infection and PAHs exposure separately and in combination on
186 biomarker responses. The mean length and weight of chubs did not differ among tanks
187 (ANOVA: $F_{(16,131)}=1.715$, $p=0.051$ and $F_{(16,131)}=1.653$, $p=0.064$, respectively). For each
188 treatment group, infection status and levels of PAHs are summarized in Table 1. Pollutant
189 concentrations were prepared from a mixed solution of 16 PAHs dissolved in cyclohexane at
190 $10 \text{ ng } \mu\text{L}^{-1}$ each, purchased from LGC standards. Although it does not give an accurate
191 representation of the fractions' compositions, the exposure setup consisted of three
192 environmentally relevant concentrations of a subset of PAHs (0.1X, 1X and 10X): 0.1X
193 concentration at $50 \text{ ng PAHs/ g of vegetal oil}$, representative of the concentration found in
194 commercial fish pellets used to feed chub during the acclimation and experimental activity;
195 1X concentration ($500 \text{ ng PAH/g vegetal oil}$), representative of PAHs levels in wild
196 invertebrate preys of chub (gammarids) captured in a river reaching good chemical status and
197 10X concentration ($5,000 \text{ ng PAHs/ g of vegetal oil}$), corresponding to the highest level of
198 PAHs quantified in wild chub (unpublished data). Serial dilutions in vegetal oil were carried
199 out before each experimental exposure and were injected using a 1-ml syringe fitted with a
200 12-cm length of 1-mm-diameter plastic tubing into the stomach of sedated chub. Fish were
201 carefully observed after each experimental contamination to control for oil regurgitation. The

202 presence of oil was confirmed in their stomachs at each sampling point and at the end of the
203 procedure, therefore the dose of PAHs diluted in vegetal oil was considered the administered
204 dose. The experimental exposures were performed on sedated fish once per week over five
205 weeks, during which all fish were measured (± 0.1 cm), weighted (± 0.5 g) and checked for
206 diseases. Before the start of the experiment, body size and weight slightly differed among
207 groups (ANOVA: $F_{(2,144)}=9.995$, $p<0.001$ and $F_{(2,144)}=10.29$, $p<0.001$, respectively), with
208 smaller and lighter fish at 1X concentration (*post-hoc tests*: all $p \leq 0.01$). No visible health or
209 behavioral changes were observed in chub during the acclimation and subsequent
210 experimental periods.

211

212 Sample collection

213 On the last week (T_5), the fish were sampled 24-h after the last PAHs exposure (Fig. 1;
214 Varanasi et al., 1989). Blood was collected from sedated fish (MS222, 80 mg L⁻¹) *via* caudal
215 vein puncture in 2.5 ml heparinized syringes and immediately centrifuged (10 min, 2,000 g,
216 4°C). The plasma supernatant was separated out and stored at -20 °C for subsequent
217 oxidative damage and anti-oxidant capacity assays. Following blood sampling, fish were
218 euthanized with an overdose of anesthetic agent (MS222, 1 g L⁻¹). Fork length (L_F ; ± 0.1 cm),
219 total weight (W_T ; ± 0.1 g) was recorded. Pelvic fins were sampled for DNA extraction. For
220 analysis of PAH bioaccumulation and biotransformation in fish, the liver was removed using
221 stainless steel instruments, weighted (W_L ; ± 0.01 g) and frozen at -20°C until further
222 processing. The Fulton's condition factor (K , $n = 158$) and the hepatosomatic index (HSI, $n =$
223 158) were calculated according to the following equations, to evaluate the host condition and
224 its metabolic efficiency, respectively:

$$225 \quad K = (W_T / L_F^3) \times 100$$

$$226 \quad \text{HSI} = (W_L / W_T) \times 100$$

227 Chub intestine and body cavity were examined for the presence of acanthocephalan parasites.
228 Whole intestine of chub were aseptically dissected. Parasites were first removed from the
229 intestine and counted to evaluate their prevalence (the percentage of infected chub) and
230 intensity (parasite number per infected host individuals, Bush et al., 1997). Microbial samples
231 ($n = 158$) were collected by scrapping away the intestinal wall with sterile scalpel blades and
232 then frozen at -80°C until DNA extraction. Note that microbial samples were free of fecal
233 material. During the course of the experiment, two fish out of the 150 chubs were found dead
234 outside their respective exposure tank, and were therefore excluded from the analysis.

235

236 Oxidative status

237 The oxidative status was evaluated by using two complementary colorimetric tests in plasma
238 samples (n = 141 for OXY and n = 134 for d-ROMs because of restricted volumes). First, d-
239 ROMs test (MC003, Diacron International, Grosseto, Italy) measures the concentration of
240 reactive oxygen metabolites (ROMs) as a measure of oxidative damage in 4 μ L plasma
241 sample. Second, to assess the antioxidant barrier of plasma, we used the OXY-Absorbent test
242 (MC435, Diacron International, Grosseto, Italy), which quantifies non-enzymatic exogenous
243 and endogenous antioxidants, after 1:100 dilution of 5 μ L of plasma sample. Calibrations
244 were achieved for both tests by measuring the absorbance (540 nm; iMark microplate reader,
245 Bio rad California, USA) of a standard solution provided with the kit. Intra-assay variation for
246 ROMs and OXY were 7.80% and 8.56%, respectively, and inter-assay variation was 7.73%
247 (ROMs) and 10.8% (OXY). OXY concentrations were expressed as mM HOCl neutralized
248 and ROMs as mM H₂O₂ equivalents.

249

250 Telomere length

251 Telomere length was determined by quantitative PCR (qPCR; BioRad CFX 96, Bio-Rad
252 USA) according to Petitjean et al. (2020), adapted for the European chub. Briefly, fin samples
253 were digested with proteinase K and DNA was extracted using the Nucleospin Tissue Kit
254 (Macherey-Nagel), following the manufacturer's instructions. DNA concentration and purity
255 were assessed with a Nanodrop ND1000 spectrophotometer (Thermo Scientific) and 35
256 samples were excluded due to poor DNA quality (n = 111). The telomere primers were
257 similar to those previously used (Petitjean et al., 2020). The control single-copy gene
258 Recombination Activating Gene 1 (RAG-1) was selected and amplified using specific primers
259 (McLennan et al., 2019) designed for the European chub: RAG1-F 5'-
260 AGAGAGAGGGGGCTAGATGA-3' and RAG1-R 5'-ATGTCAGCGAGAAGCATGG-3'.
261 qPCR was then performed using 2.5 ng of DNA per reaction. The telomere and RAG1
262 primers were respectively used at a concentration of 800 nM, and 300 nM. All samples were
263 randomly distributed across the PCR plates. Amplification efficiencies reached Mean \pm SE:
264 RAG-1, 92.36 \pm 1.10; TEL, 95.48 \pm 3.80. Telomere length (T/S ratio) was expressed relative
265 to the internal single gene control (RAG-1) and the average inter-plate variation of the T/S
266 ratio values was 2.48%.

267

268 Gut microbiota diversity - 16S rRNA gene sequencing

269 Gut microbiota samples were pooled (n = 23 pools of three individuals) based on the
270 respective exposure tanks of fish host and their infection status, to obtain enough biological
271 material to carry out DNA extraction. Microbiota samples from T₀ (n = 3) were used to
272 control for experimental conditions susceptible to affect the bacterial community (commercial
273 food), group exposed to PAH-0.1X (n = 10) to control for PAHs exposure and 10X (n = 10) to
274 evaluate the effect of both parasite infection and PAHs exposure.

275 Total DNA was extracted with a commercial kit (DNeasy PowerSoil Kit, Qiagen) according
276 to the manufacturer's instructions. DNA concentration and quality were assessed with a
277 Nanodrop spectrophotometer (NanoDrop™ 2000/2000c, Thermo Scientific) and by PCR
278 amplification of the bacterial 16S rRNA gene, respectively. The 341F/785R primers set
279 targeting the hypervariable V3-V4 region were used to amplify the bacterial 16S rRNA gene
280 fragment in the DNA extracted samples (Klindworth et al., 2013). Sequencing was performed
281 at MrDNA (www.mrdnalab.com, Shallowater, TX, USA) using the Illumina MiSeq
282 workflow. Raw sequences were demultiplexed, joined and quality-filtered using the
283 MOTHUR tool suite v1.42 (Schloss et al., 2009). Briefly, sequences shorter than 400 bp,
284 longer than 443 bp or including ambiguous base calls were discarded from the dataset.
285 Chimeric sequences were detected and removed by using the MOTHUR implementation of
286 UCHIME (Edgar et al., 2011). Following the quality filtering steps, rarefied libraries were
287 produced by randomly down-sampling to the smallest library size (11,655 reads) to correct for
288 differences in sequencing depth. Subsampling procedure led to the analysis of a total of
289 268,065 sequences, with an average length of 419 bp. Operational Taxonomic Units (OTUs)
290 were defined as groups of sequences sharing at least 97% sequence similarity. Rarefaction
291 curves, generated using the 'Vegan' package (Oksanen, 2011), indicated that the sequencing
292 depth was sufficient to cover bacterial diversity (Fig. S1). All OTUs that were observed fewer
293 than 2 times (*i.e.*, singletons) were eliminated to avoid potential artifacts in diversity
294 estimates. Last, OTUs taxonomy was determined using the SILVA release 123 ribosomal
295 RNA (rRNA) database.

296

297 Chemical analyses

298 A total of 16 parent compounds of PAH (naphthalene, acenaphthylene, acenaphthene,
299 fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, chrysene,
300 benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene,
301 dibenzo[a,h]anthracene, and benzo[ghi]perylene) and 11 hydroxylated metabolites of PAHs
302 (OH-PAH; 1-hydroxynaphthalene, 2-hydroxynaphthalene, 2-hydroxyfluorene, 3-

303 hydroxyfluorene, 9- hydroxyfluorene, 1□hydroxyphenanthrene, 2□ hydroxyphenanthrene,
304 3□ hydroxyphenanthrene, 4□ hydroxyphenanthrene, 1□hydroxypyrene, 6□hydroxychrysene,
305 and 3□hydroxybenzo[a]pyrene), listed as priority pollutants by the United States
306 Environmental Protection Agency, were quantified in fish liver (n = 127 for parent molecules
307 and n = 93 for their metabolites), following previously published protocols (Molbert et al.
308 2019). Metabolites were quantified in fewer individuals since some of them did not yield
309 sufficient biological material to carry out chemical analyses. Approximately 0.1 g of freeze-
310 dried and powdered fish liver was used for quantitative analyses of pollutants. For detailed
311 description of the analytical methods, refer to Molbert et al. 2019. Analyte concentrations
312 below the limit of quantification (LOQ) value were replaced by half of the LOQ.
313 Concentrations of parent molecules (Σ 16 PAHs) and their metabolites (Σ 11 OH-PAHs) were
314 expressed as ng g⁻¹ of dry weight (dtw).

315

316 Statistical analyses

317 All statistical models were performed in R v. 3.3.2 software (R Core Team, 2016). Analyses
318 were performed using the *lmer* statistical procedure available in the *lme4* and *lmerTest*
319 packages for linear mixed models (LMM, Pinheiro and Bates, 2000; Kuznetsova et al., 2017).
320 Variance components were estimated using a restricted maximum likelihood (REML)
321 function and all models had normal error structures. Data are presented as mean \pm standard
322 error (SE).

323 ***Question 1: Effect of parasitism on the pollutant burden in chub***

324 We tested whether parasite infection may reduce host contamination, with concentrations of
325 Σ 16 PAHs or their metabolites (Σ 11 OH-PAHs) as dependent variables, log-transformed to
326 achieve a normal distribution. Initial models included as fixed factors the effects of parasite
327 infection (two levels factor: infected or non-infected), treatment group (three levels factor:
328 Low, Medium, High), and their first-order interaction. Tank ID was treated as a random
329 effect. We used a backward elimination process to exclude non-significant variables, starting
330 with interactions, to produce minimum adequate models, providing that this resulted in a
331 reduction of the bias-adjusted Akaike's information criterion (AICc) score for small-sample
332 size. When interactions were significant, we used contrast post-hoc analyses with adjusted p-
333 values to analyse differences between groups (*emmeans* package; Lenth et al., 2017).
334 Pearson's test followed by linear models were applied to evaluate the correlation between
335 levels of PAHs in fish liver and parasite biomass.

336 ***Question 2: Effect of PAH exposure and parasitism on biomarkers and health indices?***

337 Telomere length, oxidative status (ROM and OXY) and condition indices (HSI and K) were
338 used as dependent variables. Telomere attrition is linked to normal aging in fish (Hatakeyama
339 et al., 2016). Given that telomeres shortened with fish size in this study (LM: $F_{(1,111)}=6.109$,
340 $p=0.014$; $\beta \pm \text{s.e.} = -0.036 \pm 0.014$), telomere length was first corrected with body size to
341 account for differences between treatment groups. Residuals were then used as a dependent
342 variable given their normality (Shapiro-Wilk: $p=0.107$) and homogeneity of variances
343 (Breusch-Pagan: $p=0.311$). All initial models included as fixed factors the effects of treatment
344 group, parasite infection and their first-order interaction. Tank ID was treated as a random
345 effect. Results are presented as mean parameter estimates \pm standard error ($\beta \pm \text{SE}$). We
346 performed diagnostic plots and Shapiro normality tests on residuals to check model
347 assumptions. Additionally, we checked whether telomere length (T/S ratio) was related to
348 oxidative status with Spearman correlations.

349 ***Question 3: Effect of PAH exposure and parasitism on fish bacterial diversity and***
350 ***community structure?***

351 Bacterial alpha-diversity within each pool of samples was estimated using the non-parametric
352 Shannon-Wiener's index (H'). Shifts in the structure of the digestive tract bacterial
353 community were studied using Bray Curtis dissimilarity metrics and by estimating changes in
354 the abundance of the major bacterial phyla and genera. Changes in both alpha and beta-
355 diversity among treatment groups were tested statistically by performing non-parametric
356 Kruskal-Wallis test, followed by pairwise Wilcoxon Mann-Whitney rank sum tests with
357 Bonferroni's correction. In addition, shift in bacterial community structure related to the
358 treatment group (T_0 , 0.1X and 10X) and parasite infection were assessed using permutational
359 analyses of variance (PERMANOVA).

360

361 **RESULTS**

362 At the end of the experiment, the prevalence of intestinal parasites was 69%, 80% and 67% in
363 fish exposed to 0.1X, 1X and 10X, respectively, which did not differ among treatment groups
364 ($X^2 = 3.171$, d.f.=2, $p=0.204$). The intensity of intestinal parasite did not differ among
365 treatment groups (Poisson GLM: $F_{(2,105)}=1.738$, $p = 0.181$), with a mean number of
366 *Pomphorhynchus* sp. ($\pm \text{SE}$) per infected fish of 3.47 ± 0.32 , 2.54 ± 0.35 and 2.67 ± 0.26 at
367 0.1X, 1X and 10X , respectively.

368

369 PAHs and OH-PAHs in liver

370 Analysis of fish tissues at the start of the experiment (T_0) revealed high levels of PAHs in
371 chub muscle ($n = 10$; $399 \pm 78.4 \text{ ng g}^{-1} \text{ dwt}$) and liver ($n = 5$; $784 \pm 159 \text{ ng g}^{-1} \text{ dwt}$), ten times
372 higher than the levels from a previous field study conducted at the same sampling site ($n = 6$;
373 $76 \pm 27.0 \text{ ng g}^{-1} \text{ dwt}$ in liver; Molbert et al., 2020). Mean levels of PAHs ($\pm \text{SE}$) at T_0 did not
374 significantly differ between infected ($593 \pm 131 \text{ ng g}^{-1} \text{ dwt}$, liver) and uninfected chub (1072
375 $\pm 251 \text{ ng g}^{-1} \text{ dwt}$, liver) (Fisher-Pitman permutation test: $p = 0.55$). After five-week exposure,
376 PAHs levels were significantly influenced by the interaction between infection status and
377 treatment group (LMM: $F_{(2,111.6)}=4.022$, $p=0.020$; supplementary material Table S1; Fig. 2A)
378 with higher levels of PAHs in the liver of infected chub exposed to 10X compared to infected
379 fish exposed to 0.1X (*post hoc test*: $p = 0.032$, Table S2). Although infected and uninfected
380 host did not differ in their pollutant load, levels of PAHs in parasitized chub decreased with
381 increasing parasite biomass (Pearson correlation: $r_p = -0.24$, $p = 0.028$). After 5 weeks of
382 exposure, levels of liver metabolites ($\sum 11 \text{ OH-PAHs}$) varied among treatment groups (LMM:
383 $F_{(2,14.71)}=12.08$, $p=0.001$; Table S1), with significantly higher levels in fish exposed to 10X
384 compared to 0.1X and 1X (Table S2, Fig. 2B). There was a positive correlation between
385 parent PAHs and the levels of liver metabolites (Spearman correlation: $r_s = 0.25$, $p = 0.029$).

386

387 Biomarker responses to parasite infection and PAHs contamination

388 *Condition indices* – Throughout the experiment, changes in the general condition ($\Delta_{T_0-T_5}$
389 Fulton) were negatively affected by increasing PAHs exposure (treatment, LMM:
390 $F_{(2,144)}=4.084$, $p=0.019$) but not by parasite infection (LMM: $F_{(2,144)}=0.702$, $p=0.403$). The
391 condition of chubs exposed to 0.1X significantly increased over five weeks (mean $\pm \text{SE}$, 0.035
392 ± 0.009) compared to 1X and 10X [0.003 ± 0.009 and 0.007 ± 0.008 , respectively; *post hoc*
393 *tests*: $p=0.009$ (0.1X vs. 1X), $p=0.025$ (0.1X vs. 10X); Fig. 3]. At the end of the experiment,
394 variation in Fulton's body condition (K) was not significantly explained by any variable
395 (Table S3, Fig. 4A). The hepatosomatic index (HSI) did not differ between infected and
396 uninfected chub (LMM: $F_{(1,144)}=3.093$, $p=0.08$; Tables S3 and S4) but differed among
397 treatment groups (LMM: $F_{(2,144)}=3.645$, $p=0.028$), with higher HSI at 10X concentration [*post*
398 *hoc test*: $p=0.048$ (10X vs. 0.1X), Fig. 4B].

399

400 *Oxidative status* – Variation in total plasma antioxidant capacity was not significantly
401 explained by any variable (Table S3, Fig. 4C). At the end of the experiment, variation in
402 ROM concentrations in chub plasma was explained by the interaction between infection status

403 and treatment group (LMM: $F_{(2,125.8)}=6.321$, $p=0.002$; Tables S3 and S4). Parasitized chub
404 exposed to 0.1X displayed 41% higher ROM concentrations (*post hoc test*: $p=0.007$; Fig. 4D)
405 than uninfected ones, whereas ROM concentrations in chub exposed to 10X were 39% lower
406 in infected individuals compared to uninfected ones (*post hoc test*: $p=0.021$). In addition,
407 ROM concentrations were significantly higher in parasitized chubs exposed to 0.1X compared
408 to 10X and to a lesser extent 1X [*post hoc tests*: $p=0.025$ (0.1X vs. 10X), $p=0.054$ (0.1X vs.
409 1X)]. At 1X concentration, no difference in ROM levels was observed between chubs infected
410 or not by acanthocephalan parasites (*post hoc test*: $p=0.559$).

411

412 *Telomere length* – Telomere length did not significantly differ between 0.1X, 1X and 10X
413 (T/S ratio: 1.12 ± 0.05 , 1.16 ± 0.06 and 1.04 ± 0.05 , respectively; Table S3, Fig. 4E) and was
414 not related to the infection status of chubs [T/S ratio: 1.14 ± 0.04 (infected) and 1.03 ± 0.05
415 (uninfected); Table S3]. Additionally, whatever the treatment group, there was no correlation
416 between telomere length and total antioxidant capacity of chub (Pearson correlation: $r_p \approx 0.08$,
417 all $p \geq 0.447$) nor between telomere length and ROM concentrations (Spearman correlation:
418 $r_s \approx 0.10$, all $p \geq 0.572$).

419

420 Changes in bacterial diversity and community structure

421 A total of 1,344 bacterial OTUs were detected, with an average of 183 ± 79 OTUs per sample.
422 Among the identified OTUs, 398 were exclusively found in uninfected fish samples (7.12% of
423 reads), 415 in the infected fish samples (4.84%) and 531 OTUs were detected in both infected
424 and uninfected fish samples. In addition, 159 OTUs were only detected in T₀ samples (17.2%
425 of reads), while 324 (5.40%) and 275 (2.93%) OTUs were specifically associated with 0.1X
426 and 10X samples, respectively. The Shannon-Wiener's index (H') ranged between 0.66 – 4.60
427 depending on the sample considered. Overall, samples contaminated with low or high levels
428 of PAHs had lower bacterial diversity than T₀ (Kruskal-Wallis test: $p < 0.05$) (Fig. 5A). For
429 chub exposed to PAH-0.1X, uninfected individuals harbored more diverse bacterial
430 communities than infected ones (Kruskal-Wallis test: $p < 0.05$).

431 Based on the OTUs distance matrix, the gastrointestinal bacterial community structure
432 was significantly affected by both parasite infection (PERMANOVA: $F_{\text{parasite}}= 3.1$, $p < 0.01$)
433 and PAHs exposure (PERMANOVA: $F_{\text{PAHs}}= 1.6$, $p < 0.05$). Taxonomic classification of
434 bacterial OTUs identified 6 main bacterial phyla whose relative abundance was greater than
435 1% of total reads (*i.e.*, *Proteobacteria*, *Actinobacteria*, *Tenericutes*, *Bacteroidetes*, *Firmicutes*

436 *and Fusobacteria*) (Fig. 5B). Interestingly, bacterial structural changes induced by parasite
437 infection at the OTUs level were visible at higher taxonomic levels. Fish infected by
438 *Pomphorhynchus* sp. harbored higher relative abundances in *Tenericutes* and *Fusobacteria*
439 and lower proportions of *Actinobacteria* and *Alpha-proteobacteria*, regardless of the level of
440 PAHs contamination (Table S5, Fig. 5B). The large abundance discrepancy of *Tenericutes*
441 between infected and uninfected fish is exclusively explained by the prevalence of a single
442 genus, *Candidatus bacilloplasma*, with 251 OTUs identified.

443

444 **DISCUSSION**

445 We experimentally investigated the impact of chemical pollution on host-parasite interactions,
446 by exposing naturally parasitized fish to environmental relevant levels of PAH identified as
447 general causes of the deterioration of aquatic ecosystems.

448 There was a non-significant trend towards a lower level of PAHs in infected chub
449 compared to uninfected ones. Former studies evidenced the capacity of intestinal parasites to
450 accumulate high levels of PAHs (Molbert et al., 2020; Soler-Jiménez et al., 2020) and to
451 reduce the pollutant load of their fish host (Vidal-Martínez et al., 2003; Brázová et al., 2012).
452 On the other hand, our results support the general trend reported by other authors of a
453 decrease in pollutant load in hosts when parasite biomass increases (Sures, 2002). Although
454 chubs were weekly exposed to doses of PAHs ranging from 50 to 5,000 ng g⁻¹, few significant
455 differences in pollutant load were observed among treatment groups, probably because fish
456 are able to convert up to 99% of PAHs to metabolites within 24h of the uptake (Varanasi et
457 al., 1989). PAH metabolites were indeed detected in fish tissue, with the highest levels in the
458 high treatment group (10X). Additionally, a positive correlation between parent PAH and
459 liver metabolite (OH-PAH) concentrations, ensures that PAHs-contaminated oil was absorbed
460 and biotransformed after exposure. This is further supported by the liver enlargement
461 observed in the high exposure group, which usually reflects enzyme induction (*i.e.*, an
462 increase in the rate of hepatic metabolism, as measured by the hepatosomatic index).
463 Accordingly, liver hypertrophy in fish species has been associated with PAH chronic
464 exposure or polluted environments (Everaarts et al., 1993; Larno et al., 2001).

465 Interestingly, no mortality was recorded over five-week exposure and no external or
466 internal lesions were observed, whether fish hosts were parasitized or not. In addition, very
467 few PAHs or parasite-related effects were noted on the condition factor (*K*) and
468 hepatosomatic index (HSI), which are useful tools to assess the general condition and

469 energetic status of fish. Those results suggest that environmentally relevant chemical
470 exposures combined with parasitic infection did not generate higher energetic demands nor
471 compromise the condition of the host, which concur well with previous studies (Hursky and
472 Pietrock, 2015; Lagrue and Poulin, 2015; Molbert et al., 2020). The apparent lack of
473 pathological effects at higher biological scales may be linked to the relatively low number
474 (from 1 to 11 per fish) of *Pomphorhynchus* sp. in the fish host. Indeed, intestinal parasite
475 abundance or biomass and their depth of penetration into the host tissue are the main factors
476 known to cause damage to key host organs and alter body condition (Taraschewski, 2000;
477 Maceda-Veiga et al., 2016). Additionally, the present case showed mono-infection with
478 acanthocephalans alone in the intestinal tract of chub. Importantly, at a lower biological scale,
479 exposure to both parasite infection and PAHs had an effect on the oxidative status of the host.
480 The most striking observation was that ROM concentrations significantly varied among
481 treatments groups and in relation to infection status, indicating that the effect of
482 *Pomphorhynchus* sp. on its fish host can range from negative to positive as organic pollutant
483 exposure increases. The d-ROMs test mainly measures hydroperoxides, which derive from
484 the oxidation of biomolecular substrates and act as precursors of end-products of lipid
485 peroxidation (Beaulieu and Costantini, 2014). Hydroperoxides are thus perceived as the
486 earliest markers of oxidative damage in a biological system. Secondly, total plasma
487 antioxidant activity (OXY), did not significantly differ among treatment groups, nor between
488 infected and parasitized-free individuals. Over the course of the experiment, fish were fed
489 with commercial pellets enriched in vitamins involved in the redox process. This feeding
490 protocol might have buffered the expected effect of pollutant exposure on antioxidant
491 defences given that fish were not constrained with regard to antioxidant availability. This
492 positive effect of parasites on host physiology (oxidative status), has been previously
493 attributed to the capacity of intestinal parasites to accumulate toxicants and depurate their host
494 (Morrill et al., 2019; Molbert et al., 2020), therefore reducing ROS production or activated
495 metabolites associated with chemical exposure. The outcome of host-parasite interactions may
496 thus be context dependent, with potential benefits in polluted environments (Weinersmith and
497 Earley, 2016). While the combined effect of parasitism and pollutant exposure affected the
498 oxidative status of chub, no statistically significant effects were detected on the telomere
499 length even though uninfected hosts seemed to have shorter telomere length, potentially
500 reflecting lower individual quality. Although telomeres are highly sensitive to oxidative
501 damage, which can accelerate telomere shortening (Reichert and Stier, 2017; but see
502 Boonekamp et al., 2017) we detected no associations between those two physiological

503 markers. However, considering the brief exposure to environmental stressors (5 weeks), we
504 cannot rule out that a significant relationship might have arisen over a longer experimental
505 period. Additionally, telomeres were only measured at the end of the experiment and telomere
506 length was highly variable between individuals. As a result, the large inter-individual
507 variability may have blurred the potential effect of PAHs exposure and parasite infection on
508 telomere length. For that purpose, studies should be focused on the effect of both chemical
509 exposure and parasite infection on telomere dynamics in wild animals.

510 To complete the assessment of fish health, we specifically evaluated the impact of
511 both stressors on the diversity and composition of fish gut microbiota. It is worth noting that
512 fish captivity had an impact on gut microbiota, with less diverse bacterial communities in
513 chubs at the end of the experiment compared to T₀. This significant difference may reflect the
514 change in environment and diet from natural preys to commercial food. Indeed, it has been
515 shown that diet composition modulates gut microbiota (Bolnick et al., 2014; Michl et al.,
516 2017) which, in turn, shapes intestinal immune responses (Foysal et al., 2019). While PAHs
517 exposure had no significant effect on microbial diversity, infected chubs were found to
518 harbour less diverse bacterial communities than non-infected ones. At this point, it is difficult
519 to determine whether reduced bacterial diversity is a cause or consequence of parasitic
520 infection. Indeed, it may be assumed that hosts with lower bacterial diversity might be more
521 susceptible to parasite infection (Newbold et al., 2017). Alternatively, parasites that are able
522 to colonize the intestinal tract may impair microbial communities (Ling et al., 2020), through
523 damage to gut epithelium or overlapping resource requirements (Leung et al., 2018).
524 Although negative and positive effects of parasite infection on gut microbiota has been
525 reported in the literature, there is no clear consensus about the direction of the effect (Fu et al.,
526 2019; Ling et al., 2020). In fact, given the complexity of these three-way relationships, results
527 are contingent on the studied systems (Newbold et al., 2016; Midha et al., 2017; Ling et al.,
528 2020). A more diverse gut microbiota is usually associated with « healthy » host, as bacterial
529 communities are more likely to harbour greater gene content or to be more stable in the face
530 of changing environmental conditions (*e.g.*, diet; Rinninella et al., 2019). Although the role of
531 the bacterial community in a set of metabolic functions has been demonstrated, to date no
532 single optimal diversity of gut microbiota, nor its composition, has been reported. In the
533 present study, *Proteobacteria*, *Tenericutes* and *Fusobacteria* were the main phyla detected in
534 the gut of European chubs, which is consistent with bacterial communities reported to
535 predominate in freshwater fish (Michl et al., 2017; Foysal et al 2019; Fu et al., 2019).
536 Bacterial structural changes were induced by both PAHs exposure and parasite infection, with

537 an increase in *Tenericutes* and *Fusobacteria* abundance and reduction of *Actinobacteria* in
538 infected host. A single genus, *Candidatus bacilloplasma*, solely explained the increase of
539 *Tenericutes* in parasitized fish. *C. bacilloplasma* has been identified in crustacean species, in
540 which it helps hosts to promote digestion process and up-regulate the expression of immune
541 genes (Dong et al., 2018; Foysal et al., 2019). The *Fusobacteria* also brings benefits to the
542 host through the production of butyrate, known to provide energy supply to gastrointestinal
543 cells (Collinder et al. 2003), enhance mucus production and act as an anti-inflammatory
544 (Hamer et al. 2007). Although our results provide a general overview of the taxonomic profile
545 of gut bacterial community of chub exposed to PAHs and parasites, we could not conclude on
546 the fitness consequences of the altered microbiota. Future research should seek to assess the
547 functional impact of host microbiome composition in the face of environmental pollution and
548 parasite infection, especially given that bacterial communities are able to limit chemicals
549 absorption into the small intestine, biotransform xenobiotics (e.g., PAHs) and regulate the
550 expression of major detoxification enzymes (CYP450, Li et al., 2017; Collins and Patterson,
551 2019).

552

553 **CONCLUSION**

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

564

565 **APPENDIX A. Supplementary data**

566

567 **ACKNOWLEDGMENTS**

568 This work has benefited from technical and human resources provided by
569 CEREEP-Ecotron IleDeFrance (CNRS/ENS UMS 3194) as well as financial support from the
570 Regional Council of Ile-de-France under the DIM Program R2DS bearing the references I-05-
571 098/R and 2015-1657. It has received a support under the program "Investissements d'Avenir"
572 launched by the French government and implemented by ANR with the references ANR-10-
573 EQPX-13-01 Planaqua, ANR-11-INBS-0001 AnaEE France and ANR-10-IDEX-0001-02
574 PSL.

575

576 **COMPETING INTERESTS**

577 The authors declare that they have no known competing financial interests or personal
578 relationships that could have appeared to influence the work reported in this paper.

579

580 **AUTHORS' CONTRIBUTION**

581 A.G. acquired the funding. A.G., N.M., F.P. and T.B. conceived the idea and designed the
582 methodology. A.G., F.A. and N.M. captured the fish. A.M, S.A., B.D. and N.M provided
583 animal care. N.M. carried out chemical analysis and chromatographic acquisition, ran
584 statistical analyses and drafted the manuscript. M.L.-C., N.M. and C.B. evaluated the
585 oxidative status. F.A. and C.R. performed telomere assays. T.B. and Y.C. performed DNA
586 extraction, amplification and phylogenetic analysis. All authors contributed critically to the
587 manuscript draft and gave final accordance for publication.

588

589 **FUNDING**

590 This work was financially supported by the EPHE for experimental equipment and METIS for
591 bacterial diversity analysis. N. Molbert was funded by a PhD grant from Sorbonne University
592 Environmental Transition Institute. This project was also supported by the CEPER ECONAT.
593 This work benefited from technical and human resources provided by CEREEP-Ecotron Ile-
594 de-France (CNRS/ENS UMS 3194).

595

596 **REFERENCES**

597 **Akinsanya, B., Isibor, P. O., Pentho, K. M., Mulikat, K. and Saliu, J. K.** (2019). Parasite
598 prevalence and bioaccumulation of polycyclic aromatic hydrocarbons as stressors in

599 the silver catfish, *Chrysichthys nigrodigitatus* (Siluriformes: Claroteidae). *Scientific*
600 *African* e00225. doi:10.1016/j.sciaf.2019.e00225

601 **Angelier, F., Weimerskirch, H., Barbraud, C. and Chastel, O.** (2019). Is telomere length a
602 molecular marker of individual quality? Insights from a long-lived bird. *Funct. Ecol.*
603 **33**, 1076–1087. doi:10.1111/1365-2435.13307

604 **Bagi, A., Riiser, E. S., Molland, H. S., Star, B., Haverkamp, T. H. A., Sydnes, M. O. and**
605 **Pampanin, D. M.** (2018). Gastrointestinal microbial community changes in Atlantic
606 cod (*Gadus morhua*) exposed to crude oil. *BMC Microbiology* **18**, 25.
607 doi:10.1186/s12866-018-1171-2

608 **Beaulieu, M. and Costantini, D.** (2014). Biomarkers of oxidative status: missing tools in
609 conservation physiology. *Conserv. Physiol.* **2**, No. cou014.
610 doi:10.1093/conphys/cou014

611 **Blévin, P., Angelier, F., Tartu, S., Ruault, S., Bustamante, P., Herzke, D., Moe, B., Bech,**
612 **C., Gabrielsen, G. W., Bustnes, J. O. and Chastel, O.** (2016). Exposure to
613 oxychlordan is associated with shorter telomeres in arctic breeding kittiwakes. *Sci.*
614 *Total Environ.* **563**, 125e130. doi:10.1016/j.scitotenv.2016.04.096

615 **Bolnick, D., Snowberg, L., Hirsch, P., Lauber, C. L., Org, E., Parks, B., Lusi, A. J.,**
616 **Knight, R., Caporaso, J. G. and Svanbäck, R.** (2014). Individual diet has sex-
617 dependent effects on vertebrate gut microbiota. *Nat. Commun.* **5**, 4500.
618 doi:10.1038/ncomms5500

619 **Boonekamp, J. J., Bauch, C., Mulder, E. and Verhulst, S.** (2017). Does oxidative stress
620 shorten telomeres? *Biol. Letters* **13**, 20170164. doi:10.1098/rsbl.2017.0164

621 **Brázová, T., Hanzelová, V. and Miklisová, D.** (2012). Bioaccumulation of six PCB
622 indicator congeners in a heavily polluted water reservoir in Eastern Slovakia: tissue-
623 specific distribution in fish and their parasites. *Parasitol. Res.* **111**, 779–786.
624 doi:10.1007/s00436-012-2900-3

625 **Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W.** (1997). Parasitology meets
626 ecology on its own terms: Margolis et al. revisited. *J. Parasitol.* **83**, 575–583.
627 doi:10.2307/3284227

628 **Butt, R. L. and Volkoff, H.** (2019). Gut Microbiota and Energy Homeostasis in Fish. *Front.*
629 *Endocrinol.* **10**, 9. doi:10.3389/fendo.2019.00009

630 **Canestrari, D., Bolopo, D., Turlings, T. C., Röder, G., Marcos, J. M. and Baglione, V.**
631 (2014). From parasitism to mutualism: unexpected interactions between a cuckoo and
632 its host. *Science* **343**, 1350–1352. doi:10.1126/science.1249008

- 633 **Claus, S. P., Guillou, H. and Ellero-Simatos, S.** (2017). The gut microbiota: a major player
634 in the toxicity of environmental pollutants? *Npj Biofilms and Microbiomes* **3**, 17001.
635 doi:10.1038/npjbiofilms.2017.1
- 636 **Collinder, E., Bjornhag, G., Cardona, M., Norin, E., Rehbinder, C. and Midtvedt, T.**
637 (2003). Gastrointestinal host-microbial interactions in mammals and fish: comparative
638 studies in man, mice, rats, pigs, horses, cows, elk, reindeer, salmon and cod. *Microb.*
639 *Ecol. Health Dis.* **15**, 66–78. doi:10.1080/08910600310014980
- 640 **Collins, S. L. and Patterson, A. D.** (2019). The gut microbiome: an orchestrator of
641 xenobiotic metabolism. *Acta Pharm. Sin. B* **10**, 19-32. doi:10.1016/j.apsb.2019.12.001
- 642 **De Maagd, P. G. J. and Vethaak, A. D.** (1998). *Biotransformation of PAHs and Their*
643 *Carcinogenic Effects in Fish.* In The Handbook of Environmental Chemistry Vol. 3
644 Part JPAHs and Related Compounds. Springer, Berlin, pp 265–310.
- 645 **Dong, J., Li, X., Zhang, R., Zhao, Y., Wu, G., Liu, J., Zhu, X. and Li, L.** (2018).
646 Comparative analysis of the intestinal bacterial community and expression of gut
647 immunity genes in the Chinese Mitten Crab (*Eriocheir sinensis*). *AMB Express* **8**, 192.
648 doi:10.1186/s13568-018-0722-0
- 649 **Dupoué, A., Rutschmann, A., Le Galliard, J. F., Clobert, J., Angelier, F., Marciau, C.,**
650 **Ruault, S., Miles, D. and Meylan, S.** (2017). Shorter telomeres precede population
651 extinction in wild lizards. *Sci. Rep.* **7**, 16976. doi:10.1038/s41598-017-17323-z
- 652 **Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. and Knight, R.** (2001). UCHIME
653 improves sensitivity and speed of chimera detection. *Bioinformatics* **27**, 2194–2200.
654 doi:10.1093/bioinformatics/btr381
- 655 **European Environment Agency.** (2018). Chemicals in European waters: Knowledge
656 developments. Technical report **18**. Copenhagen, Denmark.
- 657 **Evariste, L., Barret, M., Mottier, A., Mouchet, F., Gauthier, L. and Pinelli, E.** (2019).
658 Gut microbiota of aquatic organisms: A key endpoint for ecotoxicological studies.
659 *Environ. Pollut.* **248**, 989–999. doi:10.1016/j.envpol.2019.02.101
- 660 **Everaarts, J. M., Shugart, L. R., Gustin, M. K., Hawkins, W. E. and Walker, W. W.**
661 (1993). Biological markers in fish: DNA integrity, hematological parameters and liver
662 somatic index. *Mar. Environ. Res.* **35**, 101–107. doi:10.1016/0141-1136(93)90021-Q
- 663 **Filipović Marijić, V., Vardić Smrzlić, I. and Raspor, B.** (2013). Effect of acanthocephalan
664 infection on metal, total protein and metallothionein concentrations in European chub
665 from a Sava River section with low metal contamination. *Sci. Total Environ.* **463–464**,
666 772–780. doi:10.1016/j.scitotenv.2013.06.041

- 667 **Foysal, M. J., Fotedar, R., Tay, C. and Gupta, S. K.** (2019). Dietary supplementation of
668 black soldier fly (*Hermetica illucens*) meal modulates gut microbiota, innate immune
669 response and health status of marron (*Cherax cainii*, Austin 2002) fed poultry-by-
670 product and fishmeal based diets. *PeerJ* **7**, e6891. doi:10.7717/peerj.6891
- 671 **Fu, P. P., Xiong, F., Feng, W. W., Zou, H., Wu, S. G., Li, M., Wang, G. T. and Li, W. X.**
672 (2019). Effect of intestinal tapeworms on the gut microbiota of the common carp,
673 *Cyprinus carpio*. *Parasites Vectors* **12**, 252. doi:10.1186/s13071-019-3510-z
- 674 **Gaulke, C. A., Barton, C. L., Proffitt, S., Tanguay, R. L. and Sharpton, T. J.** (2016).
675 Triclosan exposure is associated with rapid restructuring of the microbiome in adult
676 zebrafish. *PLoS One* **11**, e0154632. doi:10.1371/journal.pone.0154632
- 677 **Gismond, E., Cossu-Leguille, C. and Beisel, J. N.** (2012). Acanthocephalan parasites: Help
678 or burden in gammarid amphipods exposed to cadmium? *Ecotoxicology* **21**,
679 1188–1193. doi:10.1007/s10646-012-0873-8
- 680 **Hamer, H., Jonkers, D., Troost, F. J., Bast, A., Vanhoutvin, S., Venema, K. and**
681 **Brummer, R. J.** (2007). Butyrate modulates oxidative stress in the colonic mucosa of
682 healthy humans. *Gastroenterology* **132**, A79. doi:10.1016/j.clnu.2008.11.002
- 683 **Hatakeyama, H., Yamazaki, H., Nakamura, K. I., Izumiyama-Shimomura, N., Aida, J.,**
684 **Suzuki, H., Tsuchida, S., Matsuura, M., Takubo, K. and Ishikawa, N.** (2016).
685 Telomere attrition and restoration in thenormal teleost *Oryzias latipes* are linked to
686 growth rate and telomerase activity at each life stage. *Aging* **8**, 62–75.
687 doi:10.18632/aging.100873
- 688 **Hursky, O. and Pietrock, M.** (2015). Intestinal nematodes affect selenium bioaccumulation,
689 oxidative stress biomarkers, and health parameters in juvenile rainbow trout
690 (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* **49**, 2469–2476.
691 doi:10.1021/es5048792
- 692 **Kennedy, C. R.** (2006). Ecology of the Acanthocephala. Cambridge University Press, New
693 York, New York, 249 p.
- 694 **Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner,**
695 **F. O.** (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for
696 classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.*
697 **41**, 1–11. doi:10.1093/nar/gks808
- 698 **Kuznetsova, A., Brockhoff, P. B. and Christensen R. H. B.** (2017). lmerTest Package:
699 Tests in Linear Mixed Effects Models. *J. Stat. Softw.* **82**. doi:10.18637/jss.v082.i13

700 **Lagrue, C. and Poulin, R.** (2015). Measuring fish body condition with or without parasites:
701 does it matter? *J. Fish Biol.* **87**, 836–847. doi:10.1111/jfb.12749

702 **Larno, V., Laroche, J., Launey, S., Flammarion, P. and Devaux, A.** (2001). Responses of
703 chub (*Leuciscus cephalus*) populations to chemical stress, assessed by genetic
704 markers, DNA damage and cytochrome P4501A induction. *Ecotoxicology* **10**,
705 145–158. doi: 10.1023/a:1016637809483

706 **Lenth, R., Love, J. and Hervé, M.** (2017). Package ‘emmeans’. *UnderstStat.* **34**, 216–221
707 Available from: <https://cran.r-project.org/web/packages/emmeans/index.html>.

708 **Leung, J. M., Graham, A. L. and Knowles, S. C. L.** (2018). Parasite-microbiota
709 interactions with the vertebrate gut: synthesis through an ecological lens. *Front.*
710 *Microbiol.* **9**, 843. doi:10.3389/fmicb.2018.00843

711 **Li, H. and Jia, W.** (2013). Cometabolism of microbes and host: implications for drug
712 metabolism and drug-induced toxicity. *Nature* **94**, 1-8. doi:10.1038/clpt.2013.157

713 **Ling, F., Steinel, N., Weber, J., Ma, L., Smith, C., Correa, D., Zhu, B., Bolnick, D. and**
714 **Wang, G.** (2020). The gut microbiota response to helminth infection depends on host
715 sex and genotype. *ISME J.* **14**, 1141–1153. doi:10.1038/s41396-020-0589-3

716 **Livingstone, D. R.** (2001). Contaminant-stimulated reactive oxygen species production and
717 oxidative damage in aquatic organisms. *Mar. Pollut. Bull.* **42**, 656–666.
718 doi:10.1016/s0025-326x(01)00060-1

719 **Maceda-Veiga, A., Green, A. J., Poulin, R. and Lagrue, C.** (2016). Body condition peaks
720 at intermediate parasite loads in the common bully *Gobiomorphus cotidianus*. *PLoS*
721 *One* **11**, No. e0168992. doi:10.1371/journal.pone.0168992

722 **Malaj, E., von der Ohe, P. C., Grote, M., Kuhn, R., Mondy, C. P., Usseglio-Polatera,**
723 **P., Brack, W. and Schafer, R. B.** (2014). Organic chemicals jeopardize the health
724 of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U.S.A.* **111**,
725 9549–9554. doi:10.1073/pnas.1321082111

726 **McLennan, D., Recknagel, H., Elmer, K. R. and Monaghan, P.** (2019). Distinct telomere
727 differences within a reproductively bimodal common lizard population. *Funct. Ecol.*
728 **33**, 1917–1927. doi:10.1111/1365-2435.13408

729 **Michl, S. C., Ratten, J. M., Beyer, M., Hasler, M., LaRoche, J. and Schulz, C.** (2017).
730 The malleable gut microbiome of juvenile rainbow trout (*Oncorhynchus mykiss*): Diet-
731 dependent shifts of bacterial community structures. *PLoS One* **12**, e0177735.
732 10.1371/journal.pone.0177735

- 733 **Midha, A., Schlosser, J. and Hartmann, S.** (2017). Reciprocal interactions between
734 nematodes and their microbial environments. *Front. Cell. Infect. Mi.* **7**, 144.
735 doi:10.3389/fcimb.2017.00144
- 736 **Molbert, N., Alliot, F., Santos, R., Chevreuil, M., Mouchel, J. M. and Goutte, A.** (2019).
737 Multi-residue methods for the determination of organic micropollutants and their
738 metabolites in fish tissues. *Environ. Toxicol. Chem.* **38**, 1866–1878.
739 doi:10.1002/etc.4500
- 740 **Molbert, N., Alliot, A., Leroux-Coyau, M., Médoc, V., Biard, C., Meylan, S., Jacquin, L.,**
741 **Santos, R. and Goutte, A.** (2020). Potential benefits of acanthocephalan parasites for
742 chub hosts in polluted environments. *Environ. Sci. Technol.* **54**, 5540–5549.
743 doi:10.1021/acs.est.0c00177
- 744 **Molbert, N., Angelier, F., Alliot, F., Ribout, C. and Goutte, A.** (*In revision*). Fish from
745 urban rivers and with high pollutant levels have shorter telomeres. *Biol. Letters*.
- 746 **Morrill, A., Provencher, J. F., Gilchrist, H. G., Mallory, M. L. and Forbes, M. R.** (2019).
747 Anti-parasite treatment results in decreased estimated survival with increasing lead
748 (Pb) levels in the common eider *Somateria mollissima*. *Proc. R. Soc. London, Ser. B*
749 **286**, 20191356. doi:10.1098/rspb.2019.1356
- 750 **Newbold, L. K., Burthe, S. J., Oliver, A. E., Gweon, H. S., Barnes, C. J., Daunt, F., van**
751 **der Gast, C. J.** (2016). Helminth burden and ecological factors associated with
752 alterations in wild host gastrointestinal microbiota. *ISME J.* **11**, 663–675.
753 doi:10.1038/ismej.2016.153
- 754 **Oksanen, J.** (2001). *Vegan*: Community Ecology Package. R package ver. 2.0-2.
755 [HttpCRANR-Proj](http://CRAN.R-project.org/package=vegan)
- 756 **Oluoch-Otiego, J., Oyoo-Okoth, E., Kiptoo, K. K. G., Chemoiwa, E. J., Ngugi, C. C.,**
757 **Simiyu, G., Omutange, E. S., Ngure, V. and Opiyo, M. A.** (2016). PCBs in fish and
758 their cestode parasites in Lake Victoria. *Environ. Monit. Assess.* **188**, 483.
759 doi:10.1007/s10661-016-5483-0
- 760 **Petitjean, Q., Jean, S., Côte, J., Larcher, T., Angelier, F., Ribout, C., Perrault, A.,**
761 **Laffaille, P. and Jacquin, L.** (2020). Direct and indirect effects of multiple
762 environmental stressors on fish health in human-altered rivers. *Sci. Total Environ.* **742**,
763 140657. doi:10.1016/j.scitotenv.2020.140657
- 764 **Pinheiro, J. and Bates, D.** (2000). *Mixed-Effects Models in S and S-PLUS* (1st ed). New
765 York: Springer-Verlag. Retrieved from [//www.springer.com/gp/book/9780387989570](http://www.springer.com/gp/book/9780387989570)

766 **R Core Team.** (2016). R: A language and environment for statistical computing. R
767 Foundation for Statistical Computing, Vienna, Austria.

768 **Redman, R. S., Dunigan, D. D. and Rodriguez, R. J.** (2001). Fungal symbiosis from
769 mutualism to parasitism: who controls the outcome, host or invader? *New Phytol.* **151**,
770 705–716. doi:10.1046/j.0028-646x.2001.00210.x

771 **Reichert, S. and Stier, A.** (2017). Does oxidative stress shorten telomeres in vivo? A review.
772 *Biol. Lett.* **13**, 20170463. doi:10.1098/rsbl.2017.0463

773 **Reynaud, S. and Deschaux, P.** (2006). The effects of polycyclic aromatic hydrocarbons on
774 the immune system of fish: A review. *Aquat. Toxicol.* **77**, 229–238.
775 doi:10.1016/j.aquatox.2005.10.018

776 **Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G., Gasbarrini, A. and**
777 **Mele, M.** (2019). What is the healthy gut microbiota composition? A changing
778 ecosystem across age, environment, diet, and diseases. *Microorganisms* **7**, 14.
779 doi:10.3390/microorganisms7010014

780 **Sakthivel, A., Gopalakrishnan, A. and Selvakumar, P.** (2016). Pathological manifestation
781 of the *Acanthocephalus dirus* in *Thunnus albacares*. *Asian Pac. J. Trop. Dis.* **6**, 350–
782 353. doi:10.1016/S2222-1808(15)61045-3

783 **Sánchez, M. I., Pons, I., Martínez-Haro, M., Taggart, M. A., Lenormand, T. and Green,**
784 **A. J.** (2016). When Parasites Are Good for Health: Cestode Parasitism Increases
785 Resistance to Arsenic in Brine Shrimps. *PLoS Pathog.* **12**, No. e1005459. doi:
786 10.1371/journal.ppat.1005459

787 **Santana, M. S., Sandrini-Neto, L., Neto, F. F., Oliveira Ribeiro, C. A., Di Domenico, M.**
788 **and Prodocimo, M. M.** (2018). Biomarker responses in fish exposed to polycyclic
789 aromatic hydrocarbons (PAHs): Systematic review and meta-analysis. *Environ. Pollut.*
790 **242**, 449–461. doi:10.1016/j.envpol.2018.07.004

791 **Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B.,**
792 **Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres,**
793 **B., Thallinger, G. G., Van Horn, D. J. and Weber, C. F.** (2009). Introducing
794 mothur: Open-Source, Platform-Independent, Community-Supported Software for
795 Describing and Comparing Microbial Communities. *Appl. Environ. Microb.* **75**, 7537–
796 7541. doi:10.1128/aem.01541-09)

797 **Shapiro, J. W., Williams, E. S. C. P. and Turner, P. E.** (2016). Evolution of parasitism and
798 mutualism between filamentous phage M13 and *Escherichia coli*. *PeerJ* **4**, No. e2060.
799 doi:10.7717/peerj.2060

- 800 **Silva-Gomes, A. L., Coelho-Filho, J. G., Viana-Silva, W., Braga-Oliveira, M. I., Bernardino, G.**
801 **and Costa, J. I.** (2017). The impact of *Neoechinorhynchus buttnerae* (Golvan, 1956)
802 (Eoacanthocephala: Neochinorhynchidae) *outbreaks* on productive and economic performance
803 of the tambaqui *Colossoma macropomum* (Cuvier, 1818), reared in ponds. *Lat. Am. J. Aquat.*
804 *Res.* **45**, 496–500. doi:10.3856/vol45-issue2-fulltext-25
- 805 **Sletten, S., Bourgeon, S., Bårdsen, B. J., Herzke, D., Criscuolo, F., Massemin, S., Zahn,**
806 **S., Johnsen, T. V. and Bustnes, J. O.** (2016). Organohalogenated contaminants in
807 whitetailed eagle (*Haliaeetus albicilla*) nestlings: an assessment of relationships to
808 immunoglobulin levels, telomeres and oxidative stress. *Sci. Total Environ.* **539**,
809 337e349. doi:10.1016/j.scitotenv.2015.08.123
- 810 **Snyder, S. M., Pulster, E. L. and Murawski, S. A.** (2019). Associations between chronic
811 exposure to polycyclic aromatic hydrocarbons and health indices in Gulf of Mexico
812 tilefish (*Lopholatilus chamaeleonticeps*) post *Deepwater Horizon*. *Environ Toxicol*
813 *Chem.* **38**, 2659–2671. doi:10.1002/etc.4583
- 814 **Soler-Jiménez, L. C., Hernández-Núñez, E., Velázquez-Abunader, I., Centeno-Chalé, A.**
815 **and Vidal-Martínez, V. M.** (2020). Polycyclic aromatic hydrocarbons in the cestodes
816 *Oncomegas wagneri* parasite of Mexican flounder *Cyclopsetta chittendeni*. *Parasitol*
817 *Res.* **119**, 903–913. doi:10.1007/s00436-019-06597-z
- 818 **Soltani, T., Safahieh, A., Zolgharnien, H. and Matroodi, S.** (2019). Interactions of
819 oxidative DNA damage and CYP1A gene expression with the liver enzymes in
820 Klunzinger’s mullet exposed to benzo[a]pyrene. *Toxicol. Reports* **6**, 1097-1103. doi:
821 10.1016/j.toxrep.2019.10.013
- 822 **Stauffer, J., Panda, B., Eeva, T., Rainio, M. and Ilmonen, P.** (2017). Telomere damage and
823 redox status alterations in free-living passerines exposed to metals. *Sci. Total Environ.*
824 **575**, 841e848. doi:10.1016/j.scitotenv.2016.09.131
- 825 **Sures, B. and Siddall, R.** (1999). *Pomphorhynchus laevis*: the intestinal acanthocephalan as a
826 lead sink for its fish host, chub (*Leuciscus cephalus*). *Exp. Parasitol.* **93**, 66–72.
827 doi:10.1006/expr.1999.4437
- 828 **Sures, B.** (2002). Competition for minerals between *Acanthocephalus lucii* and its definitive
829 host perch (*Perca fluviatilis*). *Int. J. Parasitol.* **32**, 1117–1122. doi:10.1016/s0020-
830 7519(02)00083-8
- 831 **Sures, B. and Siddall, R.** (2003). *Pomphorhynchus laevis* (Palaeacanthocephala) in the
832 intestine of chub (*Leuciscus cephalus*) as an indicator of metal pollution. *Int. J.*
833 *Parasitol.* **33**, 65–70. doi:10.1016/S0020-7519(02)00249-7

834 **Taraschewski, H.** (2000). Host-parasite interactions in Acanthocephala: a morphological
835 approach. *Adv. Parasitol.* **46**, 1–179. doi:10.1016/s0065-308x(00)46008-2

836 **Thielen, F., Zimmermann, S., Baska, F., Taraschewski, H. and Sures, B.** (2004). The
837 intestinal parasite *Pomphorhynchus laevis* (Acanthocephala) from barbel as a
838 bioindicator for metal pollution in the Danube River near Budapest, Hungary. *Environ.*
839 *Pollut.* **129**, 421–429. doi:10.1016/j.envpol.2003.11.011

840 **Vidal-Martínez, V. M., Aguirre-Macedo, M. L., Noreña-Barroso, E., Gold-Bouchot, G.**
841 **and Caballero-Pinzón, P. I.** (2003). Potential interactions between metazoan
842 parasites of the Mayan catfish *Ariopsis assimilis* and chemical pollution in Chetumal
843 Bay, Mexico. *J. Helminthol.* **77**, 173–184. doi:10.1079/joh2002158

844 **Varanasi, U., Stein, J. E. and Nishimoto, M.** (1989). *Biotransformation and deposition of*
845 *polycyclic aromatic hydrocarbons (PAH) in fish.* In: Varanasi, U. (Ed.), *Metabolism of*
846 *Polycyclic Aromatic Hydrocarbon in the Aquatic Environment.* CRC Press Inc., Boca
847 Raton, pp. 93–150.

848 **Wang, L. R., Wang, Y., Chen, J. W. and Guo, L. H. A.** (2009). Structure based
849 investigation on the binding interaction of hydroxylated polycyclic aromatic
850 hydrocarbons with DNA. *Toxicology* **262**, 250–257. doi:10.1016/j.tox.2009.06.015

851 **Weinersmith, K. L. and Earley, R. J.** (2016). Better with your parasites? Lessons for
852 behavioural ecology from evolved dependence and conditionally helpful parasites.
853 *Anim. Behav.* **118**, 123–133. doi:10.1016/j.anbehav.2016.06.004

854 **Whittemore, K., Vera, E., Martínez-Nevaldo, E., Sanpera, C. and Blasco, M. A.** (2019).
855 Telomere shortening rate predicts species life span. *P. Natl. Acad. Sci. USA* **116**,
856 15122–15127. doi:10.1073/pnas.1902452116

857 **Zhaoa, Y., Liub, H., Wangb, Q., Lia, B., Zhangb, H. and Pi, Y.** (2019). The effects of
858 benzo[a]pyrene on the composition of gut microbiota and the gut health of the juvenile
859 sea cucumber *Apostichopus japonicus* Selenka. *Fish Shellfish Immun.* **93**, 369–379.
860 doi:10.1016/j.fsi.2019.07.07

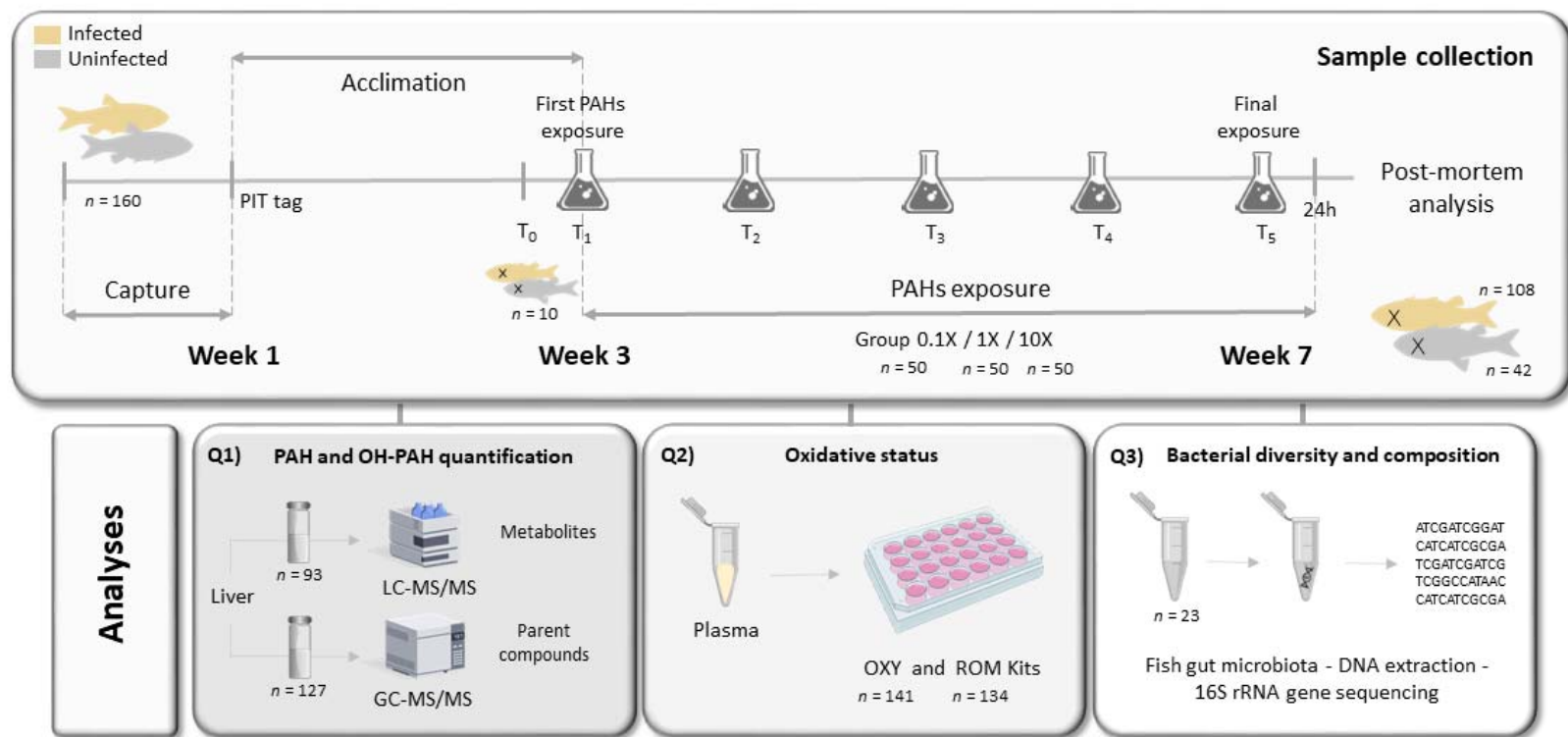
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868 **Table 1.** Biometric and biologic data (mean \pm standard error [range]) of *S. cephalus* and its
 869 intestinal parasite *Pomphorhynchus* sp. among treatment group.

Group	PAH exposure ⁽¹⁾	Infection status	n	Body weight (g)	Fork length (cm)	Parasite	
						Intensity	Biomass (g)
T₀	-	Infected	6	79.3 (\pm 13.9) [36.5 – 115]	18.8 (\pm 1.14) [15.4 – 21.8]	3.33 (\pm 0.76) [2 – 7]	0.04 (\pm 0.01) [0.034 – 0.046]
		Non-infected	4	73.5 (\pm 13.6) [47.0 – 110]	18.7 (\pm 1.03) [16.8 – 21.5]	-	-
0.1X	50 ng g ⁻¹	Infected	34	54.8 (\pm 22.1) [21.5 – 114]	16.7 (\pm 2.16) [12.3 – 21.0]	3.47 (\pm 2.23) [1 – 9]	0.04 (\pm 0.03) [0.001 – 0.12]
		Non-infected	15	55.4 (\pm 18.0) [31.0 – 79.5]	16.9 (\pm 1.91) [13.7 – 19.3]	-	-
1X	500 ng g ⁻¹	Infected	41	40.2 (\pm 18.5) [17.5 – 102]	14.9 (\pm 2.23) [11.5 – 20.9]	2.54 (\pm 2.51) [1 – 11]	0.03 (\pm 0.02) [0.005 – 0.14]
		Non-infected	9	49.7 (\pm 22.9) [24.5 – 86.0]	15.9 (\pm 2.49) [12.8 – 19.5]	-	-
10X	5000 ng g ⁻¹	Infected	33	52.2 (\pm 22.2) [13.0 – 113]	16.4 (\pm 2.18) [13.0 – 22.2]	2.67 (\pm 1.80) [1 – 7]	0.03 (\pm 0.03) [0.001 – 0.14]
		Non-infected	16	57.4 (\pm 22.4) [34.0 – 118]	16.8 (\pm 2.01) [14.1 – 22.0]	-	-

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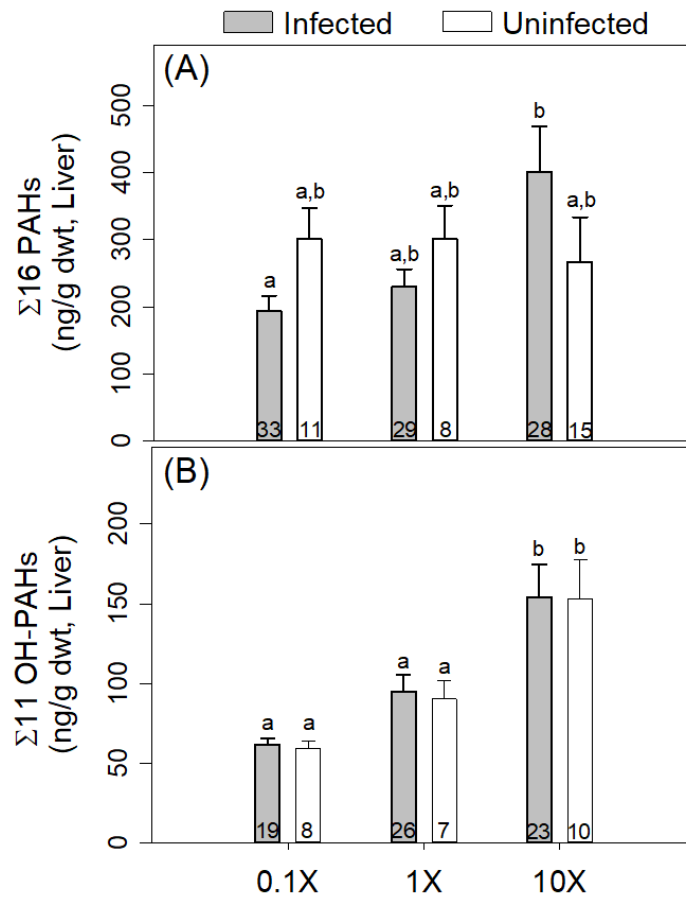
871 ⁽¹⁾ Concentrations of PAHs diluted in vegetal oil



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



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897 **Fig. 2.** Levels of (A) Σ 16 PAHs and (B) Σ 11 OH-PAHs in chub (ng g^{-1} dry weight, Liver)
 898 among treatment group (levels of PAH exposure). Numbers represent sample size. Significant
 899 differences (*post-hoc tests*: $p < 0.05$) between groups are indicated by different letters [a,b].
 900 Data are expressed as the mean \pm standard error.

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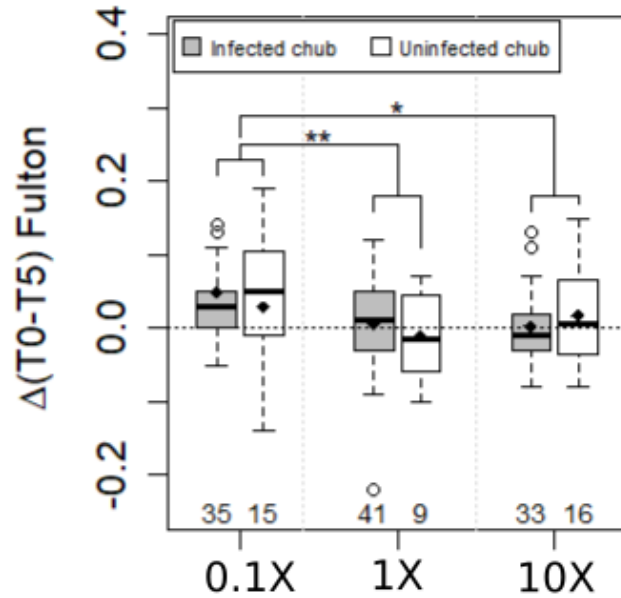
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909 **Fig. 3.** Variation of fish host general condition over five weeks of experimental PAH
 910 exposure ($\Delta_{T_0-T_5}$ Fulton), among treatment groups (levels of PAH exposure) and for infected
 911 (grey) versus uninfected hosts (white). The dotted line indicates no change in body condition.
 912 A value below the dotted line means a declining general condition of chubs after 5-week
 913 experimental exposure, and vice versa. Numbers below boxplot are sample sizes. Asterisks
 914 indicate significant effect of treatment (*: $p < 0.05$; **: $p < 0.001$).

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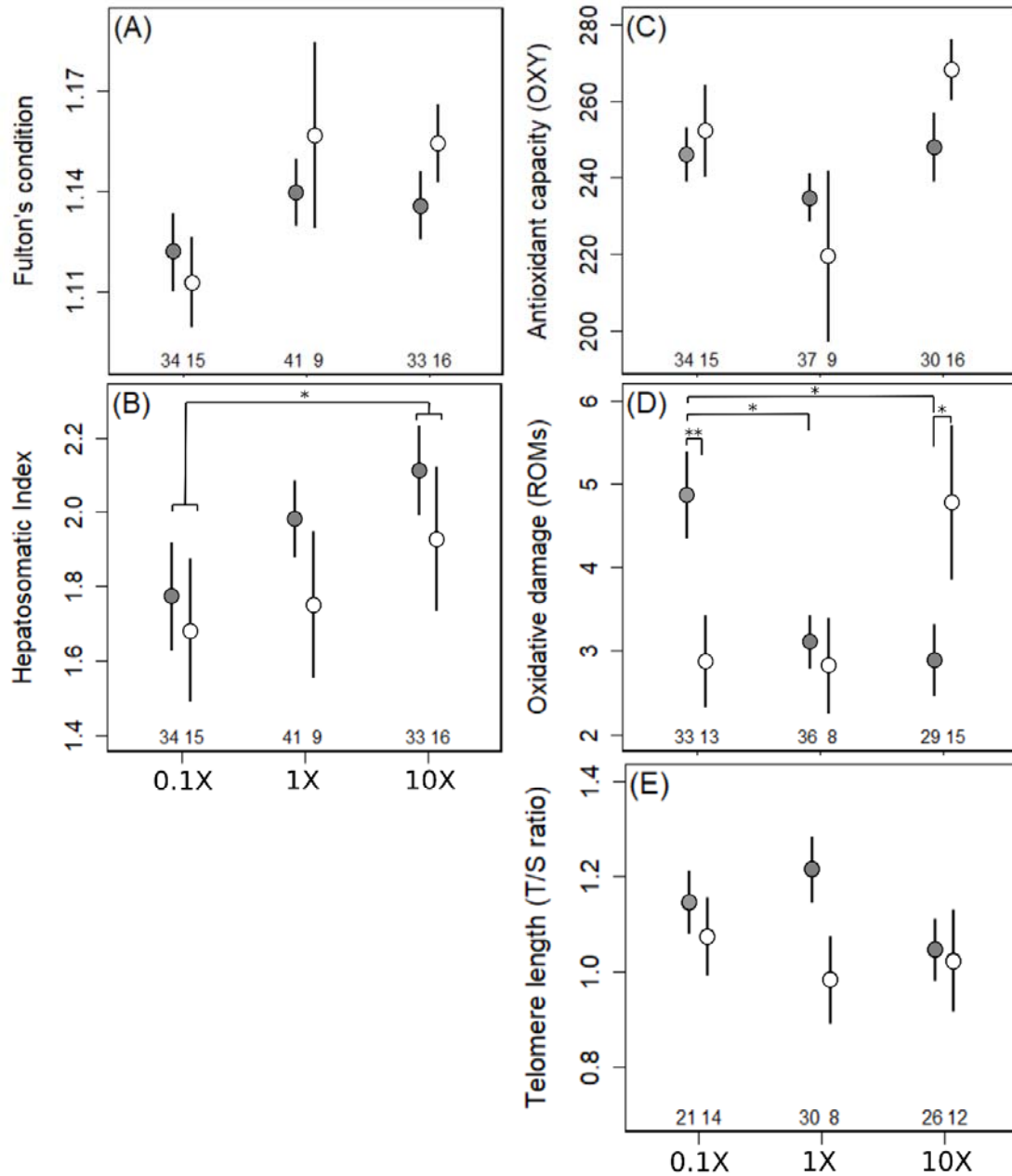
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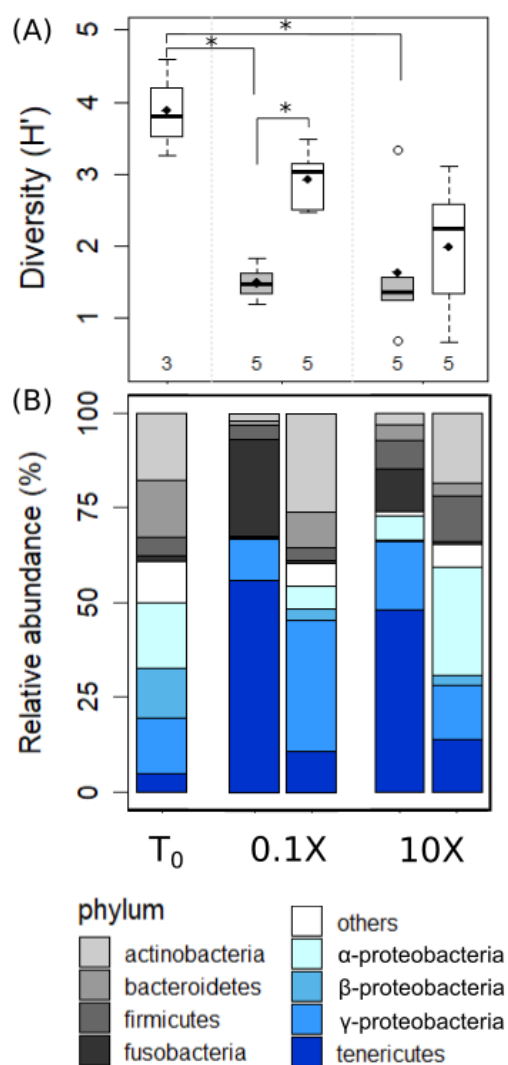
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925 **Fig. 4.** Effect of both parasite infection (grey: infected, white: uninfected) and experimental
 926 level of PAH exposure on chub (A) body condition, (B) energetic status, (C) antioxidant
 927 capacity (OXY, mM HOCl neutralized), (D) oxidative damage (ROMs, mM H₂O₂
 928 equivalents) and (E) telomere length (T/S ratio) after five-week exposure. Data are expressed
 929 as the mean \pm standard error. Numbers represent sample size. Asterisks indicate significant
 930 effect of treatment (*: $p < 0.05$; **: $p < 0.001$).

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933 **Fig. 5 (color)**. Changes in gastrointestinal bacterial diversity and community structure
 934 induced by *Pomphorhynchus* sp. infection (grey: infected, white: uninfected) and PAHs
 935 contamination. (A) Bacterial diversity estimated using the Shannon-Wiener's index (H'). (B)
 936 Distribution of the main bacterial phyla in fish gastro-intestinal tract. The *Proteobacteria*
 937 phylum was detailed for the most abundant *proteobacterial* classes (alpha, beta and gamma).
 938 The category 'others' groups minor phyla (<1% of reads) and unclassified reads. The values
 939 of the mean ± standard deviation corresponding to each phylum are summarized in Table S5.
 940 Numbers represent sample size.

5. Annex 5 – Biomarker and pollutant kinetics

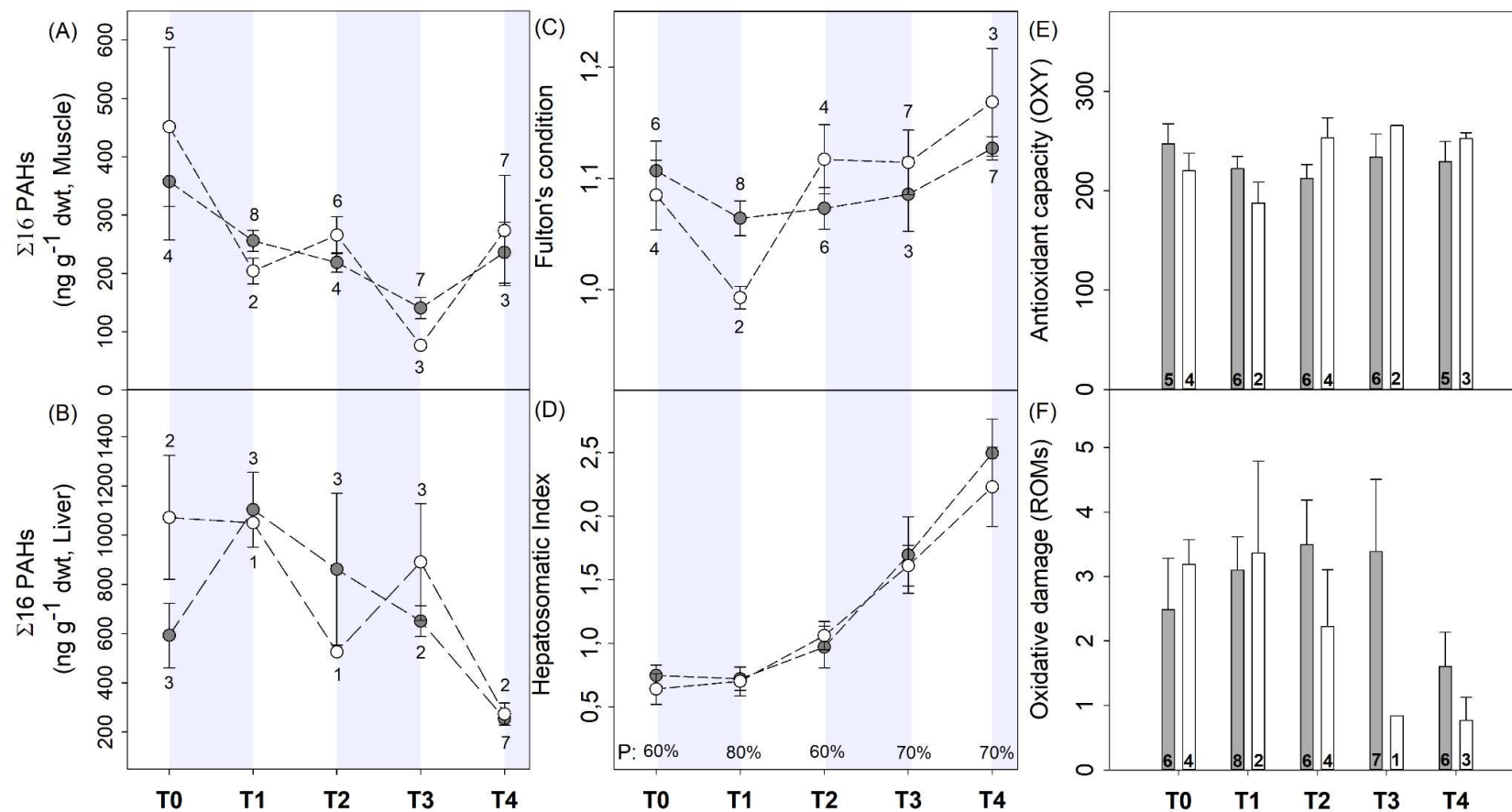


Figure. Accumulation kinetics of PAHs (ng g⁻¹ of dry weight) in muscle (A) and liver (B) and kinetics profile of oxidative stress in plasma (C-D) of infected (grey) and uninfected (white) chubs experimentally exposed to 1X of PAHs (T₁, T₂, T₃, and T₄) or not (T₀). Data are expressed as the mean ± standard error. Numbers represent sample size and P (%) represents the prevalence of intestinal parasitic infections