Title: Multiscale physiological responses to organic and inorganic pollution in the invasive mosquitofish *Gambusia holbrooki*

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Abstract

Anthropic activities often lead to the contamination of freshwater ecosystems by organic and inorganic pollutants with potential deleterious effects on wildlife health. However, some species such as the invasive mosquitofish (*Gambusia holbrooki*) can thrive in such polluted habitats, but the underpinning mechanisms are still unknown. The aim of this study was to characterize the physiological response of mosquitofish living along different pollution gradients in South of France. Eleven sites were selected according to various levels of pollutants in the water (pesticides, pharmaceuticals) and in mosquitofish tissue (PAHs, PBDEs, PCBs, organochlorines, metals). The level of the different pollutants varied among sites resulting in contrasted pollution gradients. The biological response of mosquitofish was measured using biomarkers of biotransformation, oxidative status, neurotoxicity and histopathological alteration in gills and liver. Muscle lipids, hepatosomatic condition, body condition and reproductive status were also measured. We used a Structural Equation Modelling (SEM) approach to characterize the direct and indirect effects of pollutants across biological levels. Results showed that high levels of POPs and metals affected biotransformation processes in both sexes, as well as non-enzymatic antioxidants level and resulted in gill histopathological alterations in females. In addition, pesticides increased the energetic demand reflected by reduced lipid storage in females and hepatosomatic condition in males. Interestingly, responses to pollution varied among sexes since females responded to a broader range of pollutant types than males. This study highlights some key traits underlying the tolerance to pollution of the mosquitofish, which could partly explain their invasive success in polluted ecosystems.

Key words: invasive mosquitofish, tolerance mechanisms, organic and inorganic pollutants, multiscale biological response

Highlights:

- Invasive mosquitofish are exposed to a wide range of pollutants in the wild
- Male and female exhibit different physiological responses to pollution
- POPs and metals burden affected biotransformation and gill histopathology in female
- Pesticides, metals and POPs decreased lipid storage and hepatic condition
- Pollutants did not affect reproductive status and body condition

73 ## Graphical abstract

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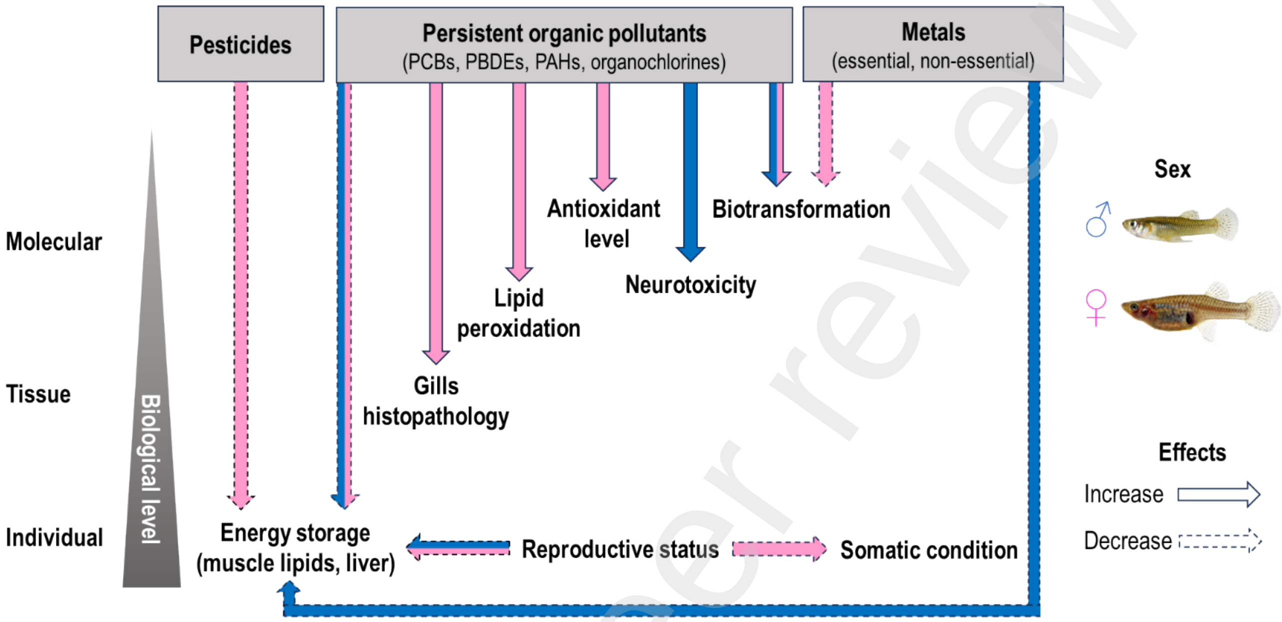

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1. Introduction

Biological responses to pollutants, from molecules to the whole organism

Chemical pollution is one of the main facets of human-driven global change that has deleterious effects on organism health and biodiversity, especially in aquatic ecosystems (Sayer et al., 2025). Indeed, an increasing number of diverse chemicals ends up in water bodies (Huang et al., 2016; Navarro et al., 2024; Wang et al., 2022; Wilkinson et al., 2022) due to agricultural, domestic and industrial activities (e.g., biocides, pharmaceuticals, metals, hydrocarbons). Fish are exposed to hundreds to thousands organic and inorganic compounds whose effects are still poorly known, especially under realistic conditions of multi-pollution in the natural environment (Hamilton et al., 2016). However, we still lack empirical studies in the wild to understand the biological effects of chronic multipollutant exposure in realistic field conditions.

To face the chemical exposome (i.e. the totality of exposure to chemical agents that individuals experience over their lives according to Wild (2005)), fish responses span from the molecular to the individual scales (Jacquin et al., 2020; Petitjean et al., 2019; Tenji et al., 2020). The first line of defense in fish exposed to pollutants is xenobiotics biotransformation and oxidative balance regulation (enzymatic and non-enzymatic antioxidant defenses) (Lushchak, 2016). The activation of biotransformation processes can produce electrophilic compounds and reactive oxygen species (ROS) leading to adverse side effects such as DNA damage and lipid peroxidation at the cellular level (van der Oost et al., 2020).

When such molecular defense mechanisms are overwhelmed, unbalanced oxidative status can impair tissular integrity (Ale et al., 2018). Histopathological alteration in key organs such as the liver or gills can disrupt organ functioning, resulting in reduced health and lifespan (Couoh-Puga et al., 2022). In addition, behavior can be altered by pollution, either indirectly through energetic demand or directly through neurotoxic effects, because neurotransmission can be affected by some organic pollutants (Fu et al., 2018; Nikhil et al., 2025; Réalis-Doyelle et al., 2024).

Ultimately, these molecular and cellular responses may generate significant energetic costs decreasing energy reserves and impairing body condition, with expected direct or indirect cascading effects on reproduction and survival. However, demonstrating the multi-scale effects of pollutants from the molecular to the individual scale remains a challenge, particularly in wild populations where fish are exposed to multiple classes of pollutants, at different concentration levels. Few studies have been able to meet this challenge (Marchand et al., 2004; Petitjean et al., 2020; Tenji et al., 2020), and further empirical works on wild species in their natural environment are needed.

Sex-dependent biological responses and strategies

The molecular to cellular responses triggered by pollutants can result in physiological and life history trade-offs (Aich et al., 2024; Cazan and Klerks, 2015; Réalis-Doyelle et al., 2023). For instance, organisms exposed to pollution can have a shortened lifespan associated to higher reproductive performance, which can maintain fitness despite physiological damage caused by pollution (Bertram et al., 2018; Bose et al., 2018; Hamilton et al., 2017). However, these adjustments associated with lifespan and reproduction outputs may differ between sex, especially in species with a strong sexual dimorphism (Evans et al., 2011). In this study, we thus explored the sex-dependent response to pollution between males and females, an issue rarely addressed in the wild.

Invasive mosquitofish as a model species in polluted environments

Invasive fish species are interesting models to understand the physiological mechanisms of adaptation to pollution because they are often tolerant to environmental perturbations, including pollution compared to local species (McCallum et al., 2014). In addition, invasive species are often characterized by a r-like strategy characterized by short generation time, high fecundity and growth rate, which can be advantageous in altered habitats (Camacho-Cervantes and Wong, 2023; Kolar and Lodge, 2001). However, the molecular and cellular characteristics sustaining such tolerance to pollution and their success in anthropized environments are still unknown.

The mosquitofish (*Gambusia holbrooki*) is an ovoviviparous fish with a short life cycle and a strong investment in reproduction (Zeng et al., 2017). It is worldwide invasive and able to live in highly polluted areas (Batty and Lim, 1999). This suggests that this species has evolved defense mechanisms and strategies to cope with these harsh conditions (Díez-del-Molino et al., 2013; Pyke, 2005). Physiological response in *Gambusia spp.* to pollutants was already studied in laboratory and field conditions (Franssen, 2011; Huang et al., 2016; Jakšić et al., 2008; Nunes et al., 2008). Mosquitofish is therefore a relevant model species to study the multiscale response to polluted environments (Huang et al., 2016), which could potentially explain its evolutionary success and invasive aspect in anthropized aquatic environment.

Objectives and hypotheses

In this study, we aimed to describe the multiscale response (from molecules to the whole organism) of male and female mosquitofish to multiple pollution patterns (polar organic compounds, POPs and metals including both metals and metalloids) using a correlative field study approach and Structural Equation Modelling analyses. We hypothesized that mosquitofish would display efficient molecular defense mechanisms in polluted sites (i.e. biotransformation capacity and antioxidant defenses), which could potentially limit molecular (lipid peroxidation) and cellular (histopathological) alterations, but at the cost of decreased energy reserves. We further hypothesized that persistent organic pollutants (POPs) will have more effects than other pollutants, due to their known toxicity and lipophilic properties. Finally, since mosquitofish exhibit a strong sexual dimorphism, we also hypothesized that responses to pollution may be sex-dependent, with lower investment in defense mechanisms in males compared to females.

2. Material and methods

2.1 Sampling design

2.1.1 Sampling sites

Eleven sites were selected in South-Eastern France along gradients of pollution (Fig.1, Tab.1). We first preselected the sampling sites based on publicly available data on (i) water and sediment pollution data from the Naïades database (eaufrance, 2022), and (ii) on the main land use and human activities surrounding sites mapped using the Corine land cover database (European Environment Agency, 2019). In addition, we selected sites where mosquitofish were present using species distribution database (GBIF, 2022). Based on this, we finally selected 11 study sites across broad gradients of multipollution linked to urban, agricultural or industrial activities to sample fish. To check the level of pollution more precisely, further detailed contamination analyses were conducted during sampling (see below).

Figure 1*: Eleven sites were selected depending on the main land use and pollution levels from public databases. Further detailed analyses of pollution levels found in the environment and fish were conducted during sampling. The main cities are underlined.*

2.1.2 Measurement of environmental parameters

Environmental parameters (Tab.1) were recorded during fish sampling. We chose environmental parameters to characterize the environmental variables potentially acting on fish physiology (Petitjean et al., 2020). Temperature (°C) and conductivity (µS) were measured using an YSI® probe. The pH was measured with a pH-meter (Metler Teledo compact®) at the laboratory directly after the sampling using water sampled in glass bottles without air to avoid pH modification. Macronutrients dissolved in the water column (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}) were analyzed using ionic chromatography (Dionex Ics-5000+ for anions; Dionex DX-120 for cations).

Table 1. *Sampling site location, sampling date and environmental characteristics: temperature (°C), specific conductivity at 25 °C (µS/cm), macronutrients concentration (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, in mg/L) in the water.*

CODE	COORDINATES X ; Y	SAMPLING DATE	TEMPERATURE	CONDUCTIVITY	PH	NO_2	NO_3	PO_4	NH_4
CAZ	3.708 ; 43.763	05/05/2022	18.2	169	6.72	0.00	0.00	0.03	0.03
PAU	3.869 ; 43.632	27/04/2022	15.5	949	7.89	0.00	0.02	0.01	0.01
LEZ	3.901 ; 43.577	16/05/2022	26.1	495	7.45	0.00	0.00	0.02	0.01
PAR	3.898 ; 43.572	14/05/2022	25.4	505	6.70	0.00	0.00	0.02	0.01
SALAM	4.002 ; 43.609	14/04/2022	17.4	754	7.76	0.01	0.42	0.01	0.09
CADAV	4.049 ; 43.608	02/05/2022	17.9	1040	8.00	0.00	0.00	0.00	0.51
LANSAM	4.075 ; 43.637	21/04/2022	14.7	851	7.71	0.02	0.54	0.10	0.19
VIS	4.214 ; 43.607	19/04/2022	17.8	958	7.82	0.00	1.46	0.07	0.02
FUM	4.682 ; 43.513	25/04/2022	14.8	1064	7.84	0.00	0.01	0.06	0.00
ARC	5.132 ; 43.505	22/06/2022	21.4	815	7.77	0.00	0.56	0.17	0.07
CADI	5.198 ; 43.414	21/06/2022	22.3	720	7.86	0.00	0.28	0.04	0.05

2.1.3 PCA on environmental parameters

A principal component analysis (PCA) was performed on physicochemical parameters to reduce dimensionality (Lê et al., 2008). The coordinate of each site on the first axis (explaining 39.7% of the total variance) was extracted and used as an estimate of global variation of abiotic environmental factors in the SEM model, to limit model over-parametrization and collinearity in subsequent models. The first PCA axis was significantly associated ($p<0.05$) with temperature, pH and conductivity (large positive values on the first axis indicated sites with low temperature and high pH and conductivity, Appendix A Fig. S1).

2.1.4 Mosquitofish sampling

Mosquitofish were collected from April to June 2022 using a landing net to capture 15 males and 15 females per site for biomarkers analyses. Individuals were euthanized on site by a lethal concentration of benzocaine according to European animal welfare guidelines. They were weighted using a precision scale (± 0.01 g) and measured with a digital caliper (± 0.1 mm). Liver, gills, brain and caudal muscle were then dissected and flash frozen in liquid nitrogen and stored at -80°C until further analyses (see section 2.3.1). Ten additional individuals per sex were transported alive to the laboratory for further precise biometric analyses and histological tissue preparation. Fish sampling and transportation were done in accordance with local authorities and French regulations. After euthanasia, mosquitofish were weighted with a precision scale (± 0.1 mg) and measured with a digital caliper (± 0.1 mm). The liver and the gonads were dissected and weighted with a precision scale (± 0.1 mg) to calculate organo-somatic indexes. The liver and the gills of the females were fixed in paraformaldehyde 4% until histopathological preparation (see section 2.3.2).

2.2 Chemical characterization

To characterized the chemical exposome of each site, different pollutant classes were targeted: pesticides, pharmaceuticals, PCBs, HAPs, PBDEs, organochlorines and metals including metalloids.

2.2.1 Organic pollution in the water column using POCIS

The protocol used to measure the concentration levels of pesticides and pharmaceuticals in the water column was fully described in Martin et al. (2025). Briefly, concentration levels were assessed in water using POCIS (Polar Organic Chemical Integrative Samplers) deployed for three weeks between May and July 2022. Twenty-nine hydrophilic pesticides and thirty-one pharmaceuticals (Supplementary Table S1) were measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and liquid chromatography coupled to high-resolution mass spectrophotometry (LC-HRMS), respectively. Time weighted average concentrations (TWAC) in water were calculated with the equation proposed by Miège et al. (2012): $TWAC_{water}=C_{pocis}.M_{pocis}/Rs.t$ where $TWAC_{water}$ corresponds to the mean concentration of the contaminant in the water (µg/L); C_{pocis} is the concentration in the POCIS (µg/g); M_{pocis} is the mass of adsorbent phase in the POCIS (g); R_s is the sampling rate (L/day) and t is the total exposure time (days).

2.2.2 POPs in whole fish tissue

We assessed persistent organic pollutants (POPs) concentrations in the whole tissue of mosquitofish since these lipophilic compounds accumulate in biological matrices. Measurements were made separately on pools of freeze-dried 20 males and 20 females, specifically collected in the same sites and same period for chemical analyses. We measured some of the priority non polar organic pollutants listed in the European water framework directive (Council of the European Communities, 2000), i.e. 15 polycyclic aromatic hydrocarbons (PAHs), 4 organochlorines (lindane, 44'DDE, penta and hexachlorobenzene), 7 polychlorinated biphenyls (PCBs: 28, 52, 101, 118, 138, 153, 180) and 6 polybrominated diphenyl ethers (PBDEs: 28, 47, 100, 99, 154, 153) according to a GC-MS/MS multiresidue method adapted and validated for fish (Molbert et al., 2019) (Supplementary Table S2). The amount of POPs in fish tissue was normalized to the total lipid content, according to published protocols (Welker and Congleton, 2005).

2.2.3 Metals in muscle tissue

The metal and metalloid concentration (µg/g dry weight, Supplementary Table S3) was assessed in caudal section including mainly muscle but also scales, skin, and vertebral bones using the same 15 males and females as for biomarkers analysis (2.1.4). Caudal sections were freeze-dried using a PowerDry LL1500 dryer and grinded individually using a QIAGEN TissueLyser II grinder. Three composite samples per site and per sex were created by pooling 5 fish together, i.e. for each site three pooled samples of males and three pooled samples of females were obtained. The samples were then digested following the procedure described in (Hansson et al., 2019), with the exception of initial sample weights and volumes used, i.e. ~25 mg of material was digested in 1 mL HNO3 (67-69%) at 90°C overnight and then diluted with mQ H2O to a 10 mL mother solution. From this, a 3 mL aliquot was then retrieved and diluted with 7 mL mQ H2O to a final 3:7 solution which was analyzed for chemical composition using an iCap triple quadruple inductively coupled mass spectrophotometry (TQ-ICP-MS) at the ICP-MS platform of the Midi-Pyrenees Observatory, Toulouse, France. Replicates, reference materials (DORM-4, DOLT-4) and blanks were used for quality checks and the results (Supplementary Table S3.1). A total of eight metals were analyzed: four non-essential metals (As, Cd, Pb, Hg) and four essential metals (Cr, Ni, Cu, Zn).

2.3 Multiscale response to pollutants

2.3.1 Molecular level

The molecular response was investigated in 15 males and 15 females per site.

The biotransformation activity was investigated in the liver. We measured the phase I CYP4501A1-dependant activity of EROD (Ethoxyresorufine-O- deethylase)(Noury, 2016) and the phase II GST activity (Glutathione-S-transferase) by colorimetric assays based respectively on Ethoxyresorufine and 1-Chloro-2,4-dinitrobenzene (CDNB) substrates (Noury, 2022).

The neurotransmission was assessed by the AChE (acetylcholine esterase) activity using colorimetric assays based on the reaction between thiocholine and the Ellman reactive DTNB (5,5′-Dithiobis[2-nitrobenzoic acid]) in the brains of males only (Noury, 2022).

Oxidative status was estimated by the measurement of the total non-enzymatic antioxidant capacity (TAC) in the liver and the oxidative damages by the measurement of malondialdehyde (MDA), a marker of lipid peroxidation in the liver for the males and in the gills for the females using commercial kits (MDA: ab118970, Abcam, Cambridge, UK; TAC: CS0790, Merck KGaA, Darmstadt, Germany).

All enzymatic and non-enzymatic levels were normalized by the amount of proteins, measured using the Bradford assay from Sigma-Aldrich (B6916) (Bradford, 1976). The measurements were made on S9 fractions obtained by grinding tissue in specific buffers (see commercial kit) using a ball mill Retsch® followed by a 9000 g centrifugation at 4 °C for 12 minutes. Reaction absorbances were obtained using a microplate reader TECAN® infinity.

Total lipid concentration (µg/mg of tissue d.w.) was quantified by sulfo-phospho-vanillin assay, reading absorbance at 525 nm and using menhaden oil in chloroform as a standard (Welker and Congleton, 2005)

2.3.2 **Histopathological alterations**

Collected tissues fixed in paraformaldehyde 4% for 24h at 4°C were rinsed in phosphate buffer saline and ethanol 70°, subsequently. Tissues were dehydrated in successive bath of ethanol graded series and then embedded in paraplast. Gill sections of 5 µm were cut using a microtome (Leica) and mounted on Menzel Gläser slides. Slides were then stained using the Masson's Trichrome staining protocol (Buzete Gardinal et al., 2019) and scanned with a Nanozoomer 2 Hamamstu.

Gill histopathological alterations were evaluated with "NDP view 2" software (version 2.9.29). The lesions were determined on 3 branchial arches per fish (Fig. 2, A). The number of epithelia lifting (gap developed between pavement cells and lamella capillaries) and lamellae fusion were counted on 10 secondary lamellae of 3 different primary lamellae. Epithelia lifting (Fig. 2, B) and lamellae fusion are considered as severe lesions (Fig. 2, C), but reversible for epithelia lifting and sometimes reversible for lamellae fusion (Bernet et al., 1999; Jacquin et al., 2019). Gill aneurysms (Fig. 2, D) is a severe pathology resulting from the collapse of the pillar cell system. It affects the vascular integrity and causes disruptions of the lamellar epithelium (Sales et al., 2017). Gill aneurysm are irreversible damages (Hassaninezhad et al., 2014). Aneurysms were counted on the whole lamellae of the 3 arches. The percentage of occurrence for each lesion was calculated for each individual as follow: 100 * mean value of the lesions counts of the 3 arches per individual / max value of all stations. With this calculation, the individual exhibiting the highest number of lesions has the maximum score of 100. The final score of histopathological alteration per individual was calculated as followed: epithelia lifting occurrence x1 (%) + lamellae fusion occurrence x2 (%) + aneurysms occurrence x3 (%). The weighting of x1, x2 and x3 respectively used for lifting, fusion and aneurysm accounts for the non-reversibility of the lesion. The results of histopathological alterations of the gills for each individual are detailed in the supplementary tables (Tab. S4).

For liver samples, sections of 4 µm-thick were cut (microtome Leica RM2255) and colored with HES (Hematoxylin Eosin Saffron) and observed under microscope (Nikon, Eclipse Ni) and a combined digital camera (DS-Ri1). Five main lesions were observed: foci of hyperplasia (cellular proliferation) (Fig. 2, E), inflammation, individual cell necrosis (dead cells) (Fig. 2, F), intra cytoplasmic inclusion reflecting cell suffering (IC inclusion sometimes called Mallory bodies) (Fig. 2, G) and anisokaryosis of hepatocytes (highly heterogeneous nuclei size corresponding to nuclear atypia) (Fig. 2, H). For each lesion, a score reflecting severity was established from 0 = normal appearance to 3 = severe alteration. A hepatic lesion score was then calculated by summing the scores of each lesion type per fish, with no ponderation according to the lesion type since the severity is taken into account in the score. The histopathologic results of the liver for each individual are detailed in the supplementary tables (Tab. S5).

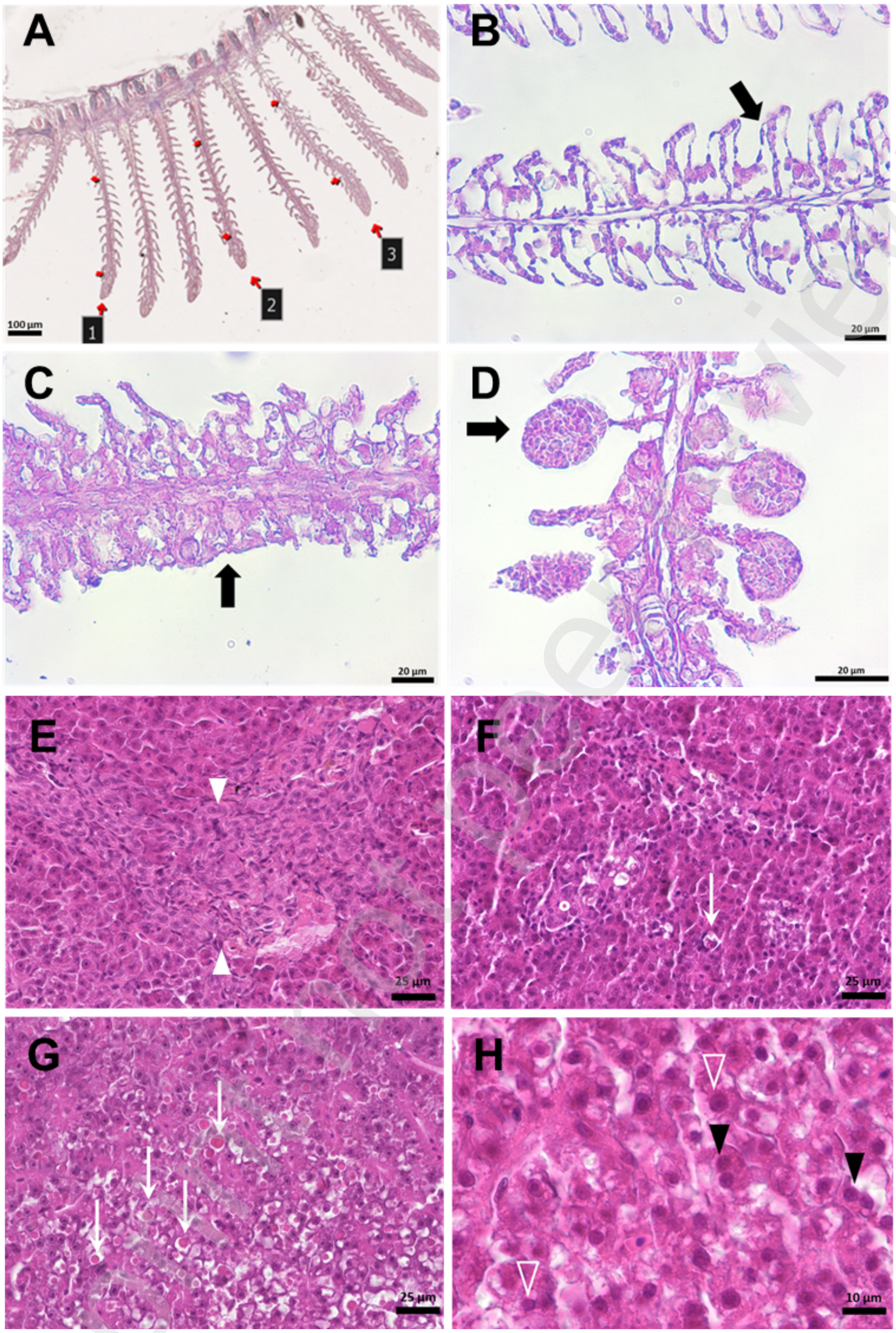

***Figure 2**: (A, B, C, D) Gills histopathological alterations recorded on female mosquitofish. A. Normal gills with an example of the counting area between two red arrows on three primary lamellae C. Epithelia lifting of the secondary lamellae (reversible and corresponding to gap developed between pavement cells and lamella capillaries). B. Fusion of the secondary lamellae (alteration sometimes irreversible). D. Aneurysm of the secondary lamellae (irreversible alteration corresponding to rupture of pillar cells). (E, F, G, H) Histopathological observation of the mosquitofish liver. E. Foci of cell proliferation corresponding to tissular hyperplasia (between arrowheads). F. Foci of inflammatory cell infiltration indicated by the white circle (°) and individual cell necrosis indicated by the white arrow. G.*

Intracytoplasmic inclusion (Mallory bodies, white arrows). H. Anisokaryosis (extreme sizes of nuclei indicated by white arrowheads) and binucleated hepatocytes (indicated by black arrowhead) corresponding to nuclear atypia.

2.3.3 Somatic and reproductive conditions

All condition and reproductive indexes were calculated using wet weight using 10 individuals per sex and per site. The body condition of male mosquitofish was measured using the Fulton index (Froese, 2006): 100 * (whole body weight / length^3), the gonad weight represented a maximum of 6% of the total body weight (present study) and was considerate as negligeable. In females, since gonad can represent up to 40% of the whole body weight (present study), the body condition was calculated as follows: 100 * (whole body weight - gonad weight) / length^3, to compare body condition without the influence of gonadal development (Alcaraz and García-Berthou, 2007; Cren, 1951). The gonadosomatic index (100 * (gonad weight / whole body weight)) and hepato-somatic index (100 * (hepatic weight / whole body weight)) were calculated to determine the reproductive status and the hepatic condition, respectively. In fish, the hepatic condition reflects both energy storage and biotransformation activity (Al-Ghais, 2013; Chellappa et al., 1995).

2.4 Statistics

Disentangling the effects of each pollutant on each response traits from correlative field approaches is a tricky issue (Grace, 2008). However, it is statistically possible to infer different causal relationship using structural equation models (SEM). SEM combine all predictors and response variables into a single linear causal model. These probabilistic models allow the investigation of direct and indirect relations among a large set of variables, which make them more appropriate than univariate analyses to study the effects of multiple predictors on multiple biological traits that are closely linked one to the other (Grace, 2008; Shipley, 2016). We used d-sep tests implemented in the piecewise SEM R-package (Lefcheck, 2021), which enables the use of generalized linear mixed models (GLMM) to build the general causal model (Shipley, 2016).

Four different SEMs were built based on four datasets, due to different sample sizes between sexes and biomarkers; one SEM model for biochemical biomarkers in males, one SEM model for biochemical biomarkers in females, one SEM for organo-somatic index in males and one SEM for histopathological alteration and organo-somatic index in females. All four models included the site identity as a random effect to account for the non-independence of mosquitofish from the same location. We built the SEMs based on a priori knowledge from the literature about causal associations between stressors and biological variables (from molecules to individuals) so as to infer relevant relationships. Furthermore, we included the standard length as a co-variable in all models to take into account the potential effects of the age on the biological variable such as biotransformation and oxidative status. We simplified SEMs using the Akaike Information Criteria (AIC) developed for d-sep test (Cardon et al., 2011); all models falling within a $\Delta AIC<2$ were considered as the best fitted. Multicollinearity was checked for all models using the variance inflation factor (VIF), and normality and the homogeneity of residuals were tested using the performance R package (Lüdecke et al., 2021). Global goodness of fit of the models was evaluated using the summary function of the piecewise SEM package including a direct separation test and a p-value. The model fit was considerate as correct when the p-value was above 0.05 (Shipley, 2016).

The effect of sex on tissular concentration of pollutants was tested using paired t test for metals. A Wilcoxon sum rank test was used to test the sex effect on tissular concentration of POPs since normality assumption were not achieved. The relation between averaged value by site of lipid concentration in muscles and the gonadosomatic index for each sex was assessed using a Spearman correlation sum rank test due to the limited available data. All statistics were performed with the R software version 4.3.0 (R Core Team, 2020) with a threshold α set to 0.05 for statistical significance tests.

3. Results

3.1 Characterization of pollution patterns

As expected, the chemical analyses of the selected sites confirmed the occurrence of contrasted gradients of pollution with different patterns across sampled sites (Fig. 3).

First, we found polar organic compounds (pesticides and pharmaceuticals) in the water column (POCIS analysis) in all study sites, with concentrations ranging from a few ng/L to µg/L for pharmaceuticals and with a maximal value around 80 ng/L for pesticides (Tab.2). Pesticides and pharmaceuticals co-occur in agricultural sites under the influence of waste water treatment plants (WWTPs) discharges, but not in the highly agricultural FUM site or in sparsely urbanized sites such as CAZ and SALAM. Two sites LANSAM and VIS had both high values for pesticides and pharmaceuticals (Fig.3A). FUM, SALAM, CAZ and PAU were mostly characterized by pesticide pollution. CADAV, CADI and ARC were mostly characterized by pharmaceuticals pollution. These results are further detailed in Martin et al. (2025).

Regarding POPs in whole fish tissue, concentration levels varied greatly among sites. The sites ARC and CADI exhibited the highest summed concentration of POPs exceeding 10 µg/g of lipids (Tab.2). The highest concentration of summed POPs by family was reached in ARC for PCBs, in PAR for PBDEs and PAHs, and in PAU for organochlorines (Fig.3B). The organochlorines were not distributed homogeneously among the sites. In particular, the PAU site was characterized by a high relative concentration of 44'-DDE concentration (Fig.3B, Supplementary Table S2). Regarding all the POPs classes analyzed (normalized to the lipid content), male and female mosquitofish exhibited no significant difference in accumulation ($p>0.05$).

Regarding metals in mosquitofish caudal muscle section, we found non-essential metals (Pb, Hg, Cd, As) and essential metals (Cr, Cu, Zn, Ni) (Fig.3C). The total concentration of essential metals mostly reflected the Zn concentration, which is an order of magnitude more concentrated than the other essential metals (Supplementary Table S3). Focusing on non-essential metals, the pattern of pollution is very different across the most polluted sites. Fish from CADAV site had the higher burden of As and moderate levels of Hg. Mosquitofish from FUM exhibited high levels of Cd and As. Mosquitofish from CAZ exhibited high levels of Pb and Cd and at SALAM, high levels of Pb and Hg. Concerning the effect of sex, males had globally higher metals burden than females, a tendency that was significant for Zn, Cd, As ($p<0.05$) and Ni ($p<0.001$) but not for Pb ($p=0.05$) (Appendix B Fig. S1).

Table 2*: Concentrations of pollutants in the different matrices: polar organic compounds in water (pharmaceuticals and pesticides in POCIS, expressed in ng/L), persistent organic compounds in whole tissue (organochlorines, PAHs, PCBs, PBDEs, expressed in ng/g of lipids) and metals in fish caudal muscle section (metals essential and non-essentials, expressed in µg/g dry weight). In this table, the POPs and metals concentrations measured in male and female pools per site were summed. Raw pollutant concentrations are given in supplementary tables (Table S1, S2, S3).*

	Sites										
Polar organic compounds	**ARC**	**CADAV**	**CADI**	**CAZ**	**FUM**	**LANSAM**	**LEZ**	**PAR**	**PAU**	**SALAM**	**VIS**
pharmaceuticals (ng/L)	528.3	531.9	498.5	1.4	34.5	1939.8	59.5	49.3	6.7	4.0	746.7
pesticides (ng/L)	7.5	20.2	11.5	15.2	52.5	87.6	14.7	8.9	7.4	4.4	35.7
Σ polar organics	535.8	552.1	510.0	16.6	86.9	2027.4	74.2	58.2	14.1	8.4	782.4
Persistent organic pollutants	**ARC**	**CADAV**	**CADI**	**CAZ**	**FUM**	**LANSAM**	**LEZ**	**PAR**	**PAU**	**SALAM**	**VIS**
Organochlorines (ng/g lipids)	125.0	199.0	121.0	2.1	118.2	464.6	219.7	284.7	4105.3	876.7	240.3
PAHs (ng/g lipids)	230.2	0.0	410.5	15.3	0.0	0.0	437.1	1109.3	82.7	633.7	42.0
PCBs (ng/g lipids)	23327.4	159.8	12988.3	31.7	838.4	746.3	1186.6	2186.8	1579.7	505.2	2948.1
PBDEs (ng/g lipids)	0.0	0.0	4.6	0.0	0.0	3.2	25.5	88.7	4.5	22.5	0.0
Σ POPs	23682.7	358.9	13524.4	49.1	956.6	1214.1	1869.0	3669.6	5772.3	2038.1	3230.4
Metals	**ARC**	**CADAV**	**CADI**	**CAZ**	**FUM**	**LANSAM**	**LEZ**	**PAR**	**PAU**	**SALAM**	**VIS**
metals essentials (µg/g dry weight)	249.4	323.8	262.1	405.9	275.9	557.8	291.4	208.0	518.9	337.0	379.5
metals non essentials (µg/g dry weight)	1.4	3.9	0.6	1.6	2.4	1.2	1.4	0.9	0.6	1.7	1.4
Σ metals	250.8	327.7	262.7	407.5	278.3	558.9	292.8	209.0	519.5	338.7	380.9

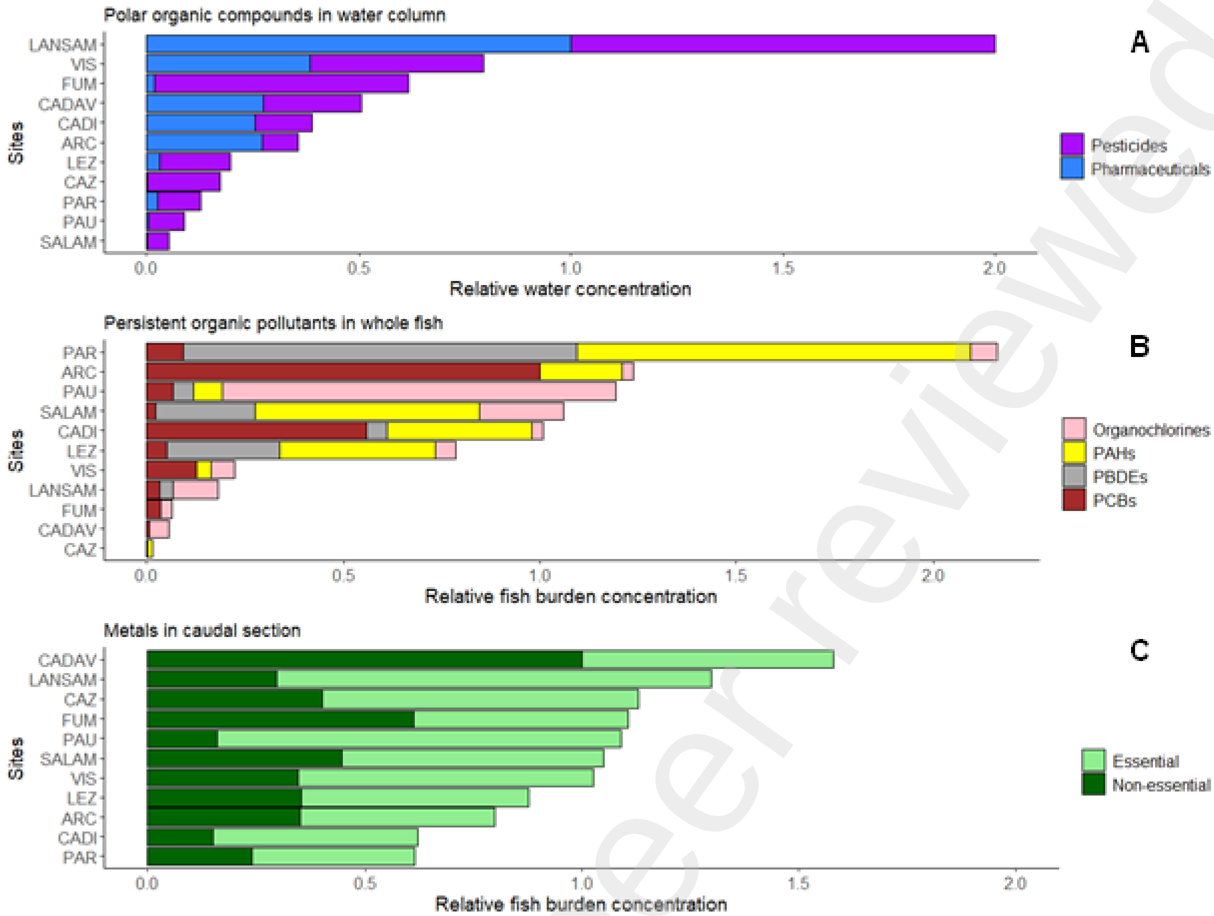

***Figure 3**: Stacked bar chart of the concentration per pollutant family for each study site: polar organic compounds in water (panel A), POPs in whole mosquitofish (panel B) and metals in mosquitofish caudal section (panel C). Data are expressed as standardized concentrations (i.e. site concentration value divided by the maximal site concentration among the dataset) for each pollutant family (pesticides, pharmaceuticals, organochlorines, PAHs, PBDEs, PCBs, essential metals, non-essential metals) in order to give the same weight to each family in this grouped representation. Concentrations from fish matrices are the summed concentrations of male and female pools. Raw pollutant concentrations are given in Tab.2 and* in supplementary tables (*Table S1, S2, S3*).

3.2 Physiological response to pollutants

Overall, the SEM models showed that all gradients of pollutant tested, except pharmaceuticals, had effects on physiological traits, but at different biological levels and in a sex-dependent manner, as detailed below.

Molecular level

As expected, mosquitofish expressed defense mechanisms at the molecular level in response to multiple pollutants, but there were striking differences between males and females (Fig. 4, Appendix C tab. S1-4). In males, few defense mechanisms were activated, whereas female mosquitofish displayed a significant molecular response to every class of pollutants, except pharmaceuticals and pesticides (Fig. 4). Specifically, in males, the main molecular response was the EROD activity (biotransformation phase I) induced by PCBs (Fig. 4A). In turn, the EROD activity was positively associated with the GST activity (biotransformation phase II) and both the EROD and GST biotransformation activity were negatively associated with the TAC level. In addition, there was a slight negative effect of PCBs on AChE neurotransmission activity. In females, we similarly found a positive association between PCBs concentration and the EROD activity. But we further found that PBDEs also triggered the EROD activity (Fig. 4B). In addition, PAHs concentration increased both GST activity and TAC level, and decreased the MDA level (Fig. 4B). Note that TAC level was also positively associated with GST activity, implying both direct and indirect effects of PAHs on the oxidative status. The oxidative status of females was also altered by essential metals and organochlorines, with essential metals increasing the TAC level and organochlorines increasing the MDA level (Fig. 4B). Finally, the EROD activity was positively associated with the GST activity -as for males-, whereas the later was negatively altered by non-essential metal concentration.

Histopathological alterations

Histopathological alterations were recorded only in females. In the liver, the level of histopathological alterations was not related to any pollutant pressure (Fig. 4B), which suggests that molecular defenses may limit hepatic damage in females. The high variability of tissular alterations in liver among sites was likely explained by the physicochemical gradient: fish living in sites with higher conductivity, pH and lower temperature had the highest lesion scores in liver (Fig. 4B). Cellular necrosis and intracytoplasmic inclusions accounted for the majority of the histopathological alterations of the liver (Appendix D Fig. S1).

Regarding the effect of pollutant on gills histopathology, the only significant association was observed in PCBs-exposed mosquitofish, with stronger gill tissular alterations for higher tissue concentration of PCBs (Fig. 4B). In the gills, aneurysm and secondary lamellae fusion were the main alterations contributing to the total gills histopathological lesion score (Appendix D Fig. S1).

Individual level: somatic condition and reproductive status

There was no link between body condition and pollution in both sexes. However, muscle lipid storage was differently affected by pollution depending on sex. In males, muscle lipid storage was not influenced by any pollutants whereas in females, PCBs and pesticides decreased lipid storage, together with physicochemical parameters (Axis 1 PCA: temperature, conductivity, pH). Body condition was positively related to the hepatosomatic index in males but not in females. In males, PBDEs and non-essential metals decreased the hepatosomatic index (Fig. 4A). In females, non-essential metals increased the hepatosomatic index.

Pollutants did not affect the reproductive status either in females nor in males (gonadosomatic index, Fig. 4 A, B). Gonadosomatic index increased with physicochemical parameters (Axis 1 PCA, characterized by higher temperature and lower conductivity and pH) both in males and females (Fig. 4B). The SEM also outlined that the reproductive status was negatively correlated to hepatosomatic index in both sexes (Fig. 4 A, B) and body condition in females.

There was an almost significant negative correlation between the gonadosomatic index and the averaged muscle lipid levels in females ($p=0.055$, Appendix E Fig. S1 A), but not in males ($p=0.8$, Appendix E Fig. S1 B).

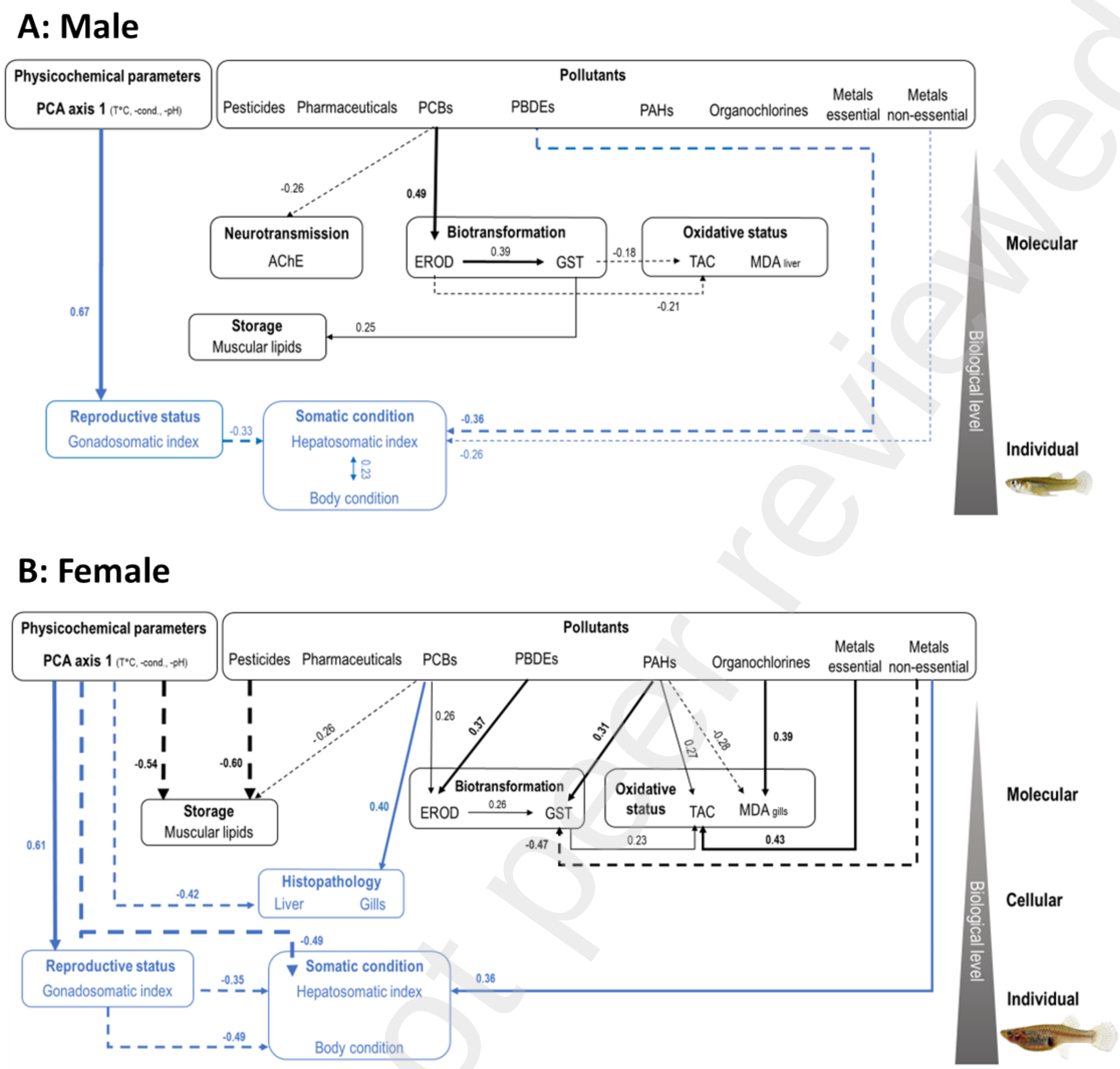

Figure 4: *Structural equation models of the multiscale physiological response to pollution and physicochemical parameters in mosquitofish. A principal component analysis (PCA) was performed on physicochemical parameters. The first PCA axis (explaining 39.7% of the total variance) was positively associated with temperature and negatively to pH and conductivity. The coordinate of each site on the first axis was extracted and used in SEMs as an estimate of physicochemical variations. Due to small body size, two different sets of individuals and two different SEMs were used for males and females and for physiological traits (black) and individual traits (blue). The two SEMs are represented on the same figure for clarity's sake. Plain arrows represent positive relationships and dashed arrows represent negative relationships. (A) Males: SEMs representation testing the direct and indirect effects of multipollution on physiological traits (black SEM: p-value=0.09, C=26.4, DF=18, N=120) and on individual traits (blue SEM: p-value=0.62, C=17.5, DF=20, N=138). (B) Females: SEMs representation testing the direct and indirect effects of multipollution on physiological traits (black SEM: p-value =0.78, C=73.6, DF=84, N=86) and on individual traits (blue SEM: C=44.31, p-value =0.37, DF=42, N=93). Standardized slope coefficients are given for each arrow. The width of the arrow is proportional to the strength (slope) of the link (fine line: <0.3, medium line: 0.3-0.5, large line: >0.5). Fish size was taken into account in models but it was not represented in the figure.*

4. Discussion

This study aimed to describe the multiscale physiological responses of mosquitofish to different patterns of pollutants gradients. Taken together, results showed that increasing levels of organic pollutants (especially POPs) and inorganic pollutants (metals) in fish tissue were associated with significant changes in biotransformation, antioxidant defenses, neurotransmission (evaluated in males only) and tissular alterations in gills (evaluated in females only). Overall, females responded to a higher number of pollutant types than males. Pollutants decreased energy reserves (muscle lipids, hepatic reserves), but mosquitofish were able to maintain their reproductive status, even in the most polluted sites, which could partly explain their invasive success in polluted environments.

4.1 Pollution patterns

A prerequisite was to characterize the chemical exposome of fish by investigating a variety of pollutant classes in the water (Martin et al., 2025) and in fish tissues. The characterization of pollution patterns highlighted a diversity of pollutant types and concentrations: pesticides, pharmaceuticals, POPs and metals, confirming that fish are chronically exposed to complex multipollution patterns. Patterns of pollution were highly contrasted among sites, and reflected surrounding human activity (agriculture, urbanization or industries). Since co-occurrence between pollutant was low, a gradient approach, testing the effect of each pollutant family, was the most appropriate in this study.

In a previous study, we previously characterized polar organic pollutants in the water (Martin et al., 2025) including in the sites in the present study among others. The present study further shows that pharmaceuticals were the predominant organic polar pollutants found in water, explained by ubiquitous discharges of treated wastewater. Pharmaceuticals of high ecotoxicological concern such as telmisartan (blood pressure regulator), tramadol (pain killer) and carbamazepine (antiepileptic) were found at concentrations up to 100 ng/L. We also found pesticides such as azoxystrobin (fungicide) and metolachlor (herbicide) in concentrations up to 33 ng/L in areas surrounded by agricultural activities.

In fish tissue, we detected significant levels of PCBs in relatively high concentrations in some of the study sites close to the Berre Lagoon, which has historically been impacted by industrial activities (Kanzari et al., 2012; Saez et al., 2008). PCBs are banned since 1987 but are persistent in the environment, especially sediments (Aravind Kumar et al., 2022). The maximum levels of PCBs found in fish tissue in our study (200 ng/g d.w.) are comparable with other French sites (Seine River) impacted by industrial activities (Azimi and Rocher, 2016), and are expected to impact fish growth and reproduction (Berninger and Tillitt, 2019). But the maximal PCBs concentrations found in mosquitofish tissue are 100 times lower than heavily polluted sites in the United States (New Bedford, Nacci et al., 2010), inducing adaptive resistance in killifish (*Fundulus heteroclitus*).

We also found high levels of PAHs in fish from the freshwater marina Port Arianne (PAR), likely because of boat traffic. In particular, we found high concentrations of benzo[a]pyrene (BaP, up to 9 ng/g d.w. in males), which is one of the most toxic PAHs with known genotoxic, mutagenic, and teratogenic effects (Zheng et al., 2016). This is consistent with BaP concentrations in sediments at this site (over 1 mg/Kg d.w. in 2013, eaufrance, 2022). As a comparison, this is 3-fold higher than the median value of 0.3 mg/Kg d.w. in the Seine fluvial estuary, which was classified as having medium concentration (GIP Seine-aval, 2008). We also found high levels of PBDEs in mosquitofish from the PAR station, with a maximum concentration of 68 ng/g of lipid in females. However, this concentration can be considered as low compared to the maximal levels reported in fish worldwide (16,300 ng/g lipid in USA, La Guardia et al., 2024).

We also found essential and non-essential metals in fish tissues at different levels depending on sampling sites. The highest level of As was found in the CADAV site (up to 1.7 µg/g d.w.), probably due to the proximity of the mesohaline Or lagoon. In fact, As tissue concentration generally increases in marine organisms as it is highly bioconcentrated in marine algae and then enter in the fish diet compared to freshwater ecosystems where As is mainly present in inorganic form and taken up by gills and epithelia (Azizur Rahman et al., 2012). These levels are however lower than fish living in the Tusket River (Canada) with historical metal contamination (up to 5.5 µg/g w.w. in muscles) (Foley et al., 2022). Elevated levels of Cd (up to 0.005 µg/g d.w.) were also found in mosquitofish from the FUM site located in a rice field drainage channel from Camargue, which is likely due to agricultural pollution by drainage waters from surrounding rice crops (Xia et al., 2024). This Cd muscle concentration is also lower than the Tusket River (up to 0.2 µg/g w.w. in muscles) (Foley et al., 2022). In addition, we found high levels of zinc in some sites (up to 400 µg/g d.w.), probably due to the proximity to agricultural activities and WWTPs discharges (Davis et al., 2001). Such Zn muscle concertation in muscles are expected to trigger adverse biological effects on fish (Giardina et al., 2009).

Interestingly, males had a significantly higher tissue concentrations of Zn, Ni, Cd and As than females. While some previous studies reported no sex effect (Adeogun et al., 2020; Burger et al., 2003; Varol et al., 2022), others found sex differences with lower Cd and Cu concentrations in the liver of females compared to males, possibly explained by the transfer of metals from the liver to the gonads during vitellogenesis (Nikolić et al., 2021). The higher metal concentrations in males could also be explained by lower dilution due to slower growth in males (Kurtul et al., 2022; Merciai et al., 2014).

Overall, we thus found complex patterns of organic and inorganic multipollution across study sites, at concentrations expected to impact mosquitofish physiology and health.

4.2 Multiscale response of mosquitofish to pollutants

All families of pollutants, except pharmaceuticals, induced a significant biological response in males and/or females. At the individual level, body condition and reproductive status were not directly influenced by pollution, but rather by water physicochemical parameters, including temperature. At the molecular level however, several defense mechanisms were induced by pollutants, more strongly in females compared to males.

4.2.1 Biotransformation and antioxidant molecular defenses

Several pollutants affected biotransformation biomarkers as expected from the literature (Schlenk et al., 2024). SEM models showed that exposure to PCBs increased CYP1A-dependent EROD activity in liver in males and females. In females, several other pollutants increased biotransformation processes: PBDEs increased EROD activity and PAHs increased GST activity. This result is consistent with the fact that hepatic biotransformation is one of the first barriers against tissular accumulation of organic pollutants, as demonstrated in other fish species (Santana et al., 2018; Whyte et al., 2000). This concentration-dependent induction of biotransformation processes with POPs suggest that mosquitofish exposed to moderate PAHs and PCBs exposure did not exhibit genetic downregulation of AhR pathway, as documented in killifish species (*Fundulus spp.*) living in heavily polluted environments by PCBs and PAHs (Franco et al., 2022; Oziolor et al., 2019; Whitehead et al., 2017). In addition, we found a positive association between EROD and GST activity in both sexes. This is consistent with the known cross-activation between phase I and II biotransformation activities (Zhang et al., 2009). Moreover, non-essential metals decreased GST activity in females. This finding is consistent with previous studies showing that GST activity can be negatively regulated by non-essential metals such as Pb, Cd, As and Hg by lowering the concentration of the GST co-substrate (reduced glutathione) (Dobritzsch et al., 2020).

In addition, in females, the oxidative status was directly affected by PAHs, organochlorines and essential metals. In details, PAHs increased the total non-enzymatic antioxidant capacity (TAC). TAC levels were also indirectly affected by pollutants through increased biotransformation activity in males and females. Since metabolization of pollutants in liver generates ROS and reactive metabolites (Lushchak, 2016), non-enzymatic antioxidants are essential to balance the level of pro-oxidants and limit adverse effects (Halliwell, 1996; Parvez and Raisuddin, 2006).

Overall, females were strongly affected by several organic and inorganic pollutants, which triggered crossed biotransformation and antioxidant processes. In males, fewer defense responses were observed. This is consistent with previous studies highlighting sex-dependent antioxidant responses to pollution (Piazza et al., 2024).

4.2.2 Molecular and cellular damage

Most previous studies show that pollutants can cause lipid peroxidation and increased MDA levels when antioxidant defenses are overwhelmed (Gutteridge and Halliwell, 1990). In our study, only organochlorines induced higher gill MDA levels (evaluated in females only), suggesting that antioxidant defenses are sufficient to prevent lipid damage by other pollutant types.

Regarding neurotoxicity, inhibition of AChE activity was observed in the brain of males with higher PCBs burden. It would be interesting to confirm this relation in females in another study. As reviewed by (Fu et al., 2018), AChE activity is primarily known to be impaired by organophosphate and carbamate pesticides. However, we did not find significant relation between pesticides levels and AChE in this study. According to Fu et al. (2018), other organic compounds such as PCBs also exhibit neurotoxic properties on AChE. Since AChE activity is essential for neurotransmission, its dysregulation by pollutants may have consequences on mosquitofish behavior, as shown in arctic charr (Réalis-Doyelle et al., 2023).

In females, we examined the potential tissular damage caused by pollutants (Yancheva, 2016). PCBs triggered severe histological alterations of the gills. The observation of tissular lesions in wild populations exposed to PCBs may potentially reveal strong repercussions for health, since gills are involved in key physiological functions such as ion regulation, gas exchange and immunity (Evans et al., 2005; Sales et al., 2017). In a further study, it would be informative to characterize molecular defense mechanisms in the gills.

In the liver however, we found no association between pollutants and hepatic alterations, suggesting that defense mechanisms activated in the liver may limit hepatic tissular alterations. Interestingly, the histopathological status of the liver was mainly influenced by other environmental factors (temperature, conductivity, pH).

4.2.3 Somatic condition and reproductive status

Overall, the reproductive status and the body condition were not affected by pollutants. The gonadosomatic index was rather affected by physicochemical parameters, likely driven by temperature, that influences gonadal development in this species (Vondracek et al., 1988). In addition, hepatosomatic index decreased with increased gonadosomatic index in both sexes, suggesting energy reallocation towards reproduction. In females, body condition also decreased as the gonadosomatic index increased, highlighting that reproduction is particularly costly for females as shown in previous studies (Chung et al., 2021; Weeks, 1996). These results are consistent with the reproductive strategy observed in other invasive species, where the available energy is mainly allocated to reproduction (Nepal et al., 2024; Vondracek et al., 1988).

We also found that pesticides and PCBs decreased lipid storage in female muscles (but not in males), suggesting a reallocation of lipids to fuel physiological defenses. Another potential explanation is that pollution could alter lipid storage capacity (pollution-induced lipogenesis or lipolysis) as shown in previous studies (Dreier et al., 2020; Pierron et al., 2007; Zheng et al., 2014).

Regarding lipid storage, we found contrasted physiological responses to pollution between sexes. In males, we did not find any effects of pollution on muscle lipid levels, contrary to females. This may be because reproduction is less costly for males compared to females in ovoviviparous species (Moffett et al., 2022), that have to fuel the embryo larval growth (Saleh-Subaie et al., 2021). Accordingly, we found a negative relationship between lipid reserves and gonadosomatic index in females, but not in males. In our study, we also found that lipid levels in females decreased with increasing temperature, which is concordant with the increase in reproductive investment with temperature.

Our hypothesis of a divergence between males and females in terms of energy compromise in response to pollutants is further illustrated regarding the liver condition. Contrary to females, PBDEs and non-essential metals decreased the hepatosomatic index in males. The higher burden of non-essential metals in males may explain this sex-dependent negative effect. But this result may also suggest that males mobilize hepatic energy substrates (mainly composed of glycogen according to Chellapa et al. (1995)) rather than muscle lipids, illustrating that males and females may not mobilize the same pools of energy.

Taken together, all these results show that multipollution activates molecular defenses but at the cost of decreased muscle lipids and/or hepatic reserves, which may potentially affect other fitness-related traits that were not investigated in the present study.

4.3 Benefits and limits of correlative environmental studies

Since pollution and invasive species represent two major threats to aquatic biodiversity (Geist and Hawkins, 2016; Kumar et al., 2024), advancing our understanding of invasive species response to pollution is important to better anticipate ecological impacts on aquatic biodiversity. Using an empirical approach, we described the physiological consequences of environmental gradients of multipollution on mosquitofish across biological scales. However, we are conscious that we cannot extrapolate these results beyond the observed range of pollutant level. Moreover, correlative studies are potentially biased by a snapshot effect, since the most affected individuals may have been counter-selected and/or long-term toxicant exposure can result in acquired resistance, as described in heavily polluted sites by PCBs an PAHs in the killifish (Reid et al., 2016; Whitehead et al., 2017). Moreover, environmental studies have to deal with the interactions between pollutants and other natural factors. This is a limitation because effects of pollutants on organisms can differ depending on abiotic environmental conditions (Holmstrup et al., 2010; Laskowski et al., 2010). Accordingly, we used SEM approach to decipher the effect of each environmental predictors on response variables. Our sampling design did not allow the assessment of crossed-effects between pollutants. Such studies required replicated sites with pollutants present alone and in combination compared to control sites without pollutants, which is a real challenge in environmental studies, especially in mixed-use catchments.

Despite these limitations, our study provides strong evidence that mosquitofish can face multiple types of pollutants, by activating molecular defenses. This correlative study also shows that mosquitofish are able to maintain their reproductive status, even in the most polluted sites, which could partly explain their invasive success. Other reproductive parameters, such as fecundity or offspring success, should be assessed to refine the assessment of the effects of pollution on mosquitofish reproduction. However, investigating such reproductive traits is challenging in wild mosquitofish populations, since reproduction is characterized by multiple reproductive cycles over a breeding season with strong environmental effects of temperature and photoperiod (Pyke, 2005; Vondracek et al., 1988). In field studies with spot sampling of individuals, the number of eggs per female or sexual maturity cannot be used as reliable proxies of reproductive effort, unless population dynamics is accurately monitored by frequent cohort sampling, as previously done in *G. holbrooki* along salinity gradient (Alcaraz and García-Berthou, 2007; Ruiz-Navarro et al., 2011). To better estimate and predict the long-term effects of chemical stressors and the consequences at populational level, multicohort and multigenerational studies are needed to assess fitness-related variables such as growth, lifespan, fecundity or reproductive outputs.

5. Conclusion

This field study showed that wild mosquitofish are exposed to mixture of past legacy and emerging pollutants, triggering multi-scale biological effects. Our study also outlined that exposure to pollutants (especially organochlorines pesticides, PCBs and non-essential metals) has a physiological cost: activation of molecular defense mechanisms, histopathological alteration of gills, mobilization of muscle lipids and hepatic reserves. But, overall, mosquitofish are able to maintain an active reproduction, even in the most polluted sites. Our study also revealed contrasting responses to pollution between males and females, suggesting different trade-off regarding defense strategies. From a biomonitoring point of view, our study suggests that female mosquitofish may be more informative than males, as the females activate molecular responses to a broader panel of pollutants. This finding adds further information on the sex-dependent biological responses to stressors in a highly invasive species living in harsh environments.

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Ethics. Fish sampling was accorded by the local authority (Hérault, Gard, Bouche du Rhône departmental direction of territories). Fish handling and euthanasia were carried out in compliance with animal welfare rules, in accordance with European guidelines (Annexes III and IV of Directive 2010/63/EU revised in 2023).

CRediT authorship contribution statement. Martin Nicolas: Writing – original draft, Investigation, Data curation, Formal analysis, Visualization. Blanchet Simon: Writing – review & editing, Supervision, Formal analysis. Hermet Sophie: Writing – review & editing, Investigation. Larcher Thibaut: Writing – review & editing, Investigation. Gros Romain: Investigation. Hansson Sophia Veronica: Writing – review & editing, Investigation, Data curation. Elena Gomez: Investigation, Writing – review & editing, Data curation. Geoffroy Duporté: Investigation, Writing – review & editing, Data curation. Andrés Sauvêtre: Investigation, Writing – review & editing, Data curation. Goutte Aurélie: Investigation, Writing – review & editing, Data curation. Bénédicte Lalot: Investigation, Data curation. Jean Séverine: Writing – review & editing. Jacquin Lisa: Writing – review & editing, Supervision. Farcy Emilie: Writing – review & editing, Conceptualization, Methodology, Investigation, Data curation, Supervision, Funding acquisition.

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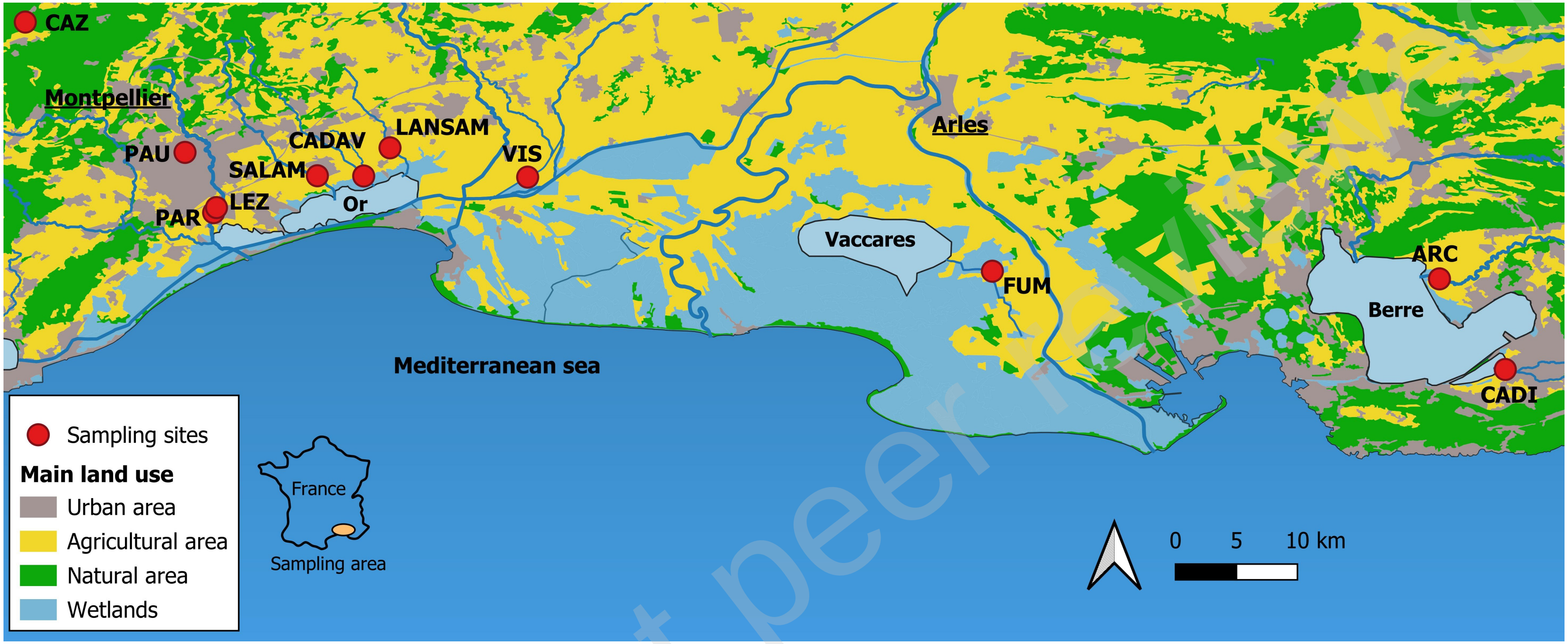
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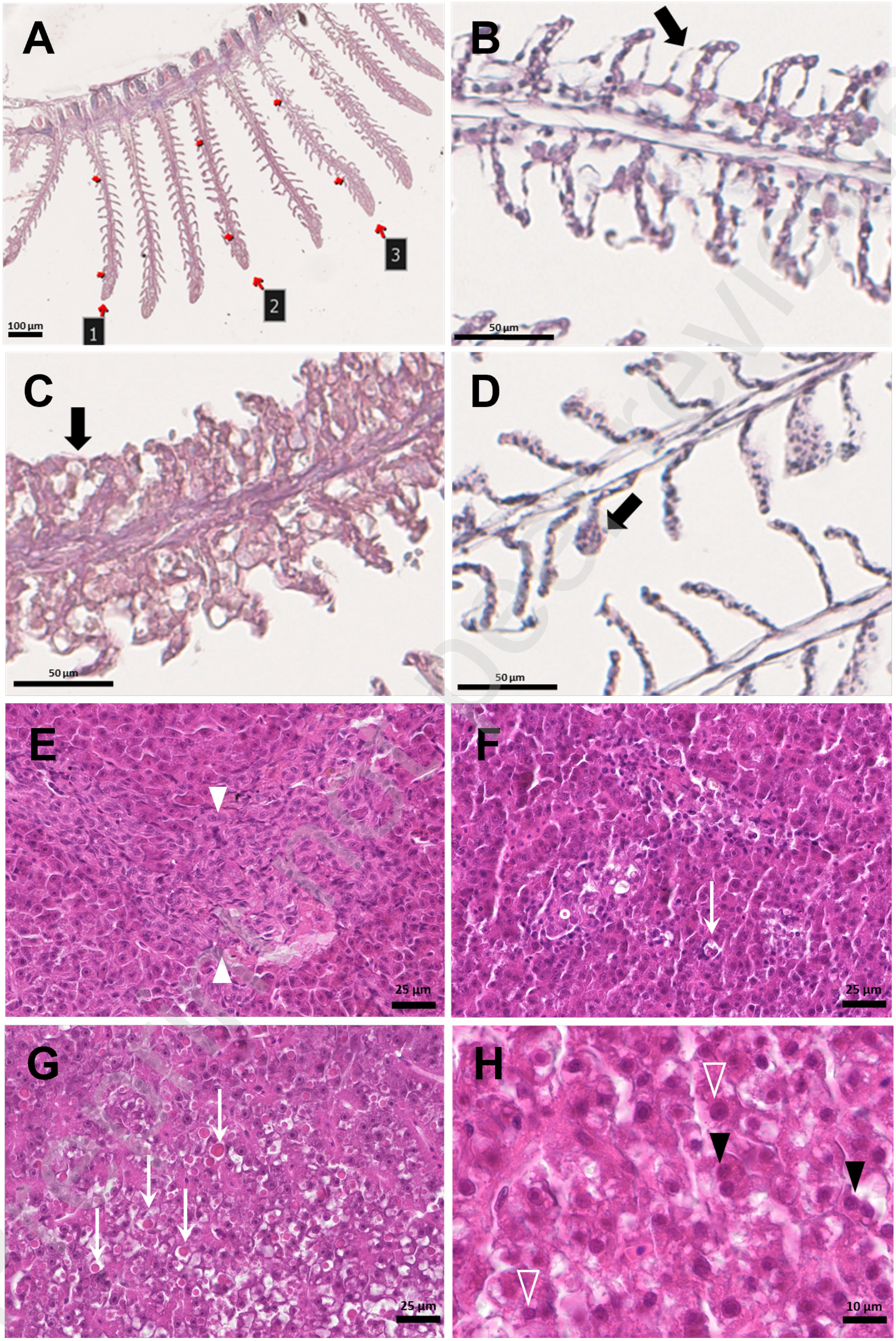
A
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2
3
100 µm
B
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D
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E
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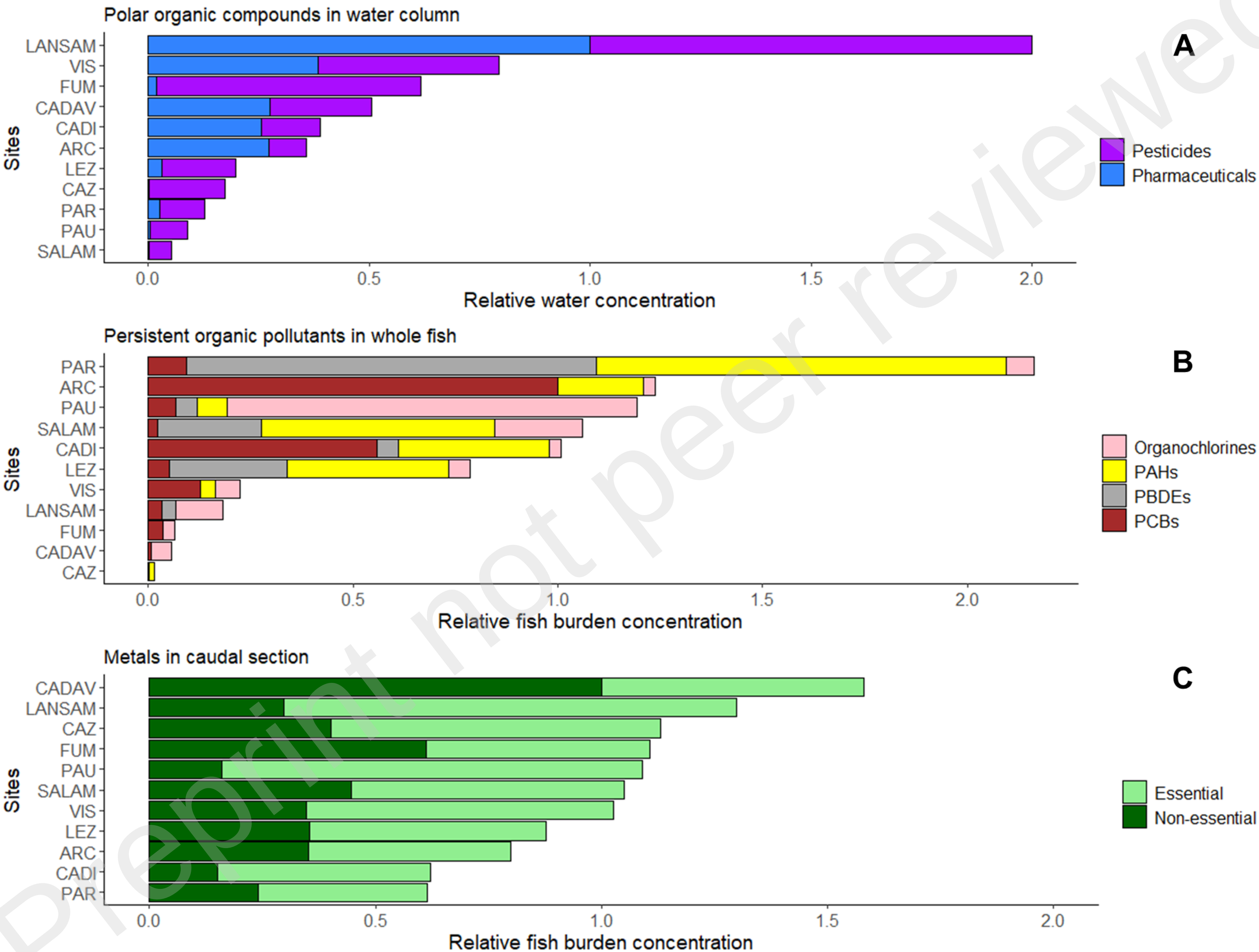
Polar organic compounds in water column
A
Pesticides
Pharmaceuticals
LANSAM
VIS
FUM
CADAV
CADI
ARC
LEZ
CAZ
PAR
PAU
SALAM
Sites
0.0
0.5
1.0
1.5
2.0
Relative water concentration
Persistent organic pollutants in whole fish
B
Organochlorines
PAHs
PBDEs
PCBs
PAR
ARC
PAU
SALAM
CADI
LEZ
VIS
LANSAM
FUM
CADAV
CAZ
Sites
0.0
0.5
1.0
1.5
2.0
Relative fish burden concentration
Metals in caudal section
C
Essential
Non-essential
CADAV
LANSAM
CAZ
FUM
PAU
SALAM
VIS
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Sites
0.0
0.5
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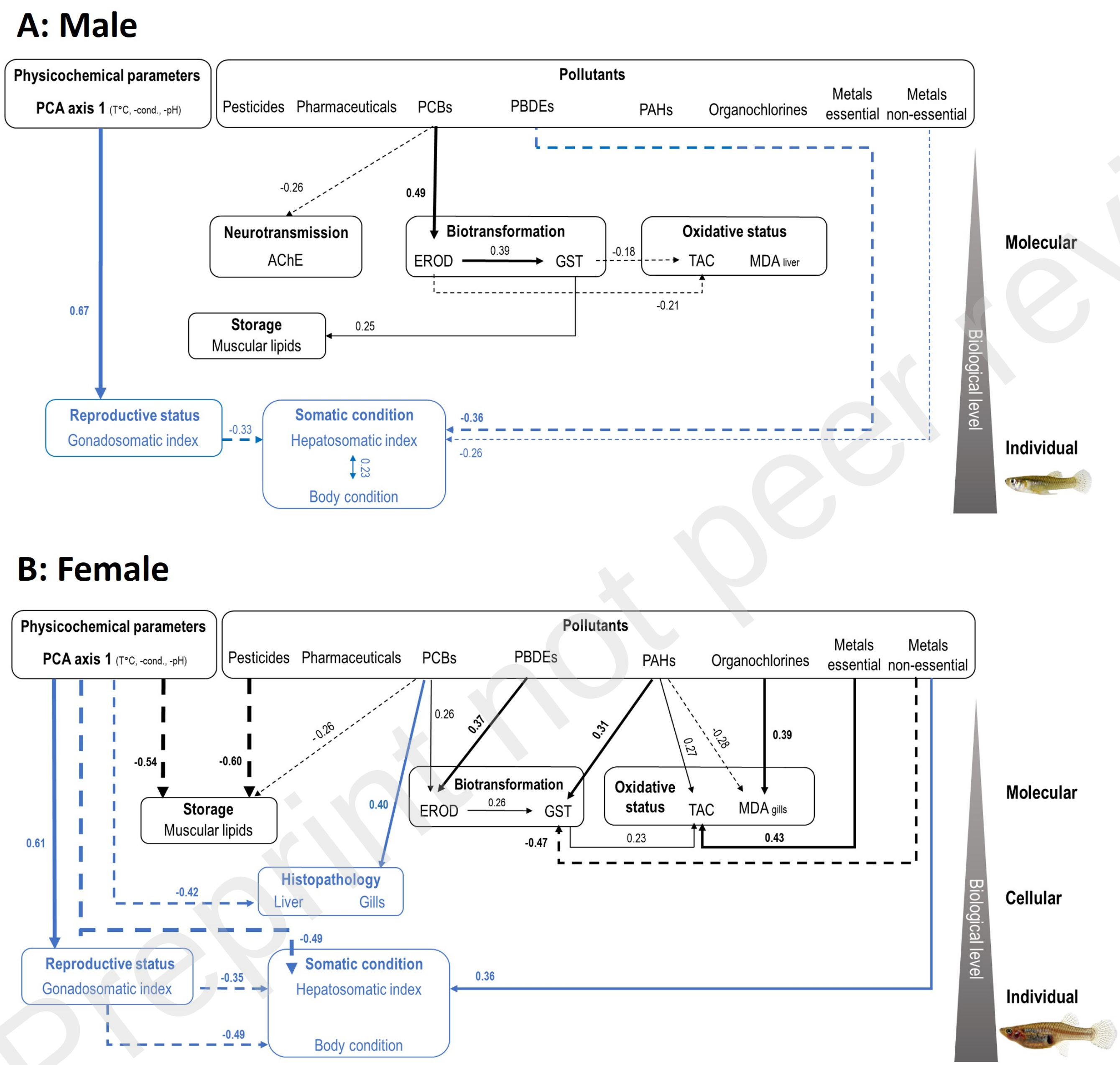
Relative fish burden concentration



A: Male
Physicochemical parameters
PCA axis 1 (T°C, -cond., -pH)
Pollutants
Pesticides
Pharmaceuticals
PCBs
PBDEs
PAHs
Organochlorines
Metals essential
Metals non-essential
-0.26
0.49
Neurotransmission
AChE
Biotransformation
EROD
0.39
GST
-0.18
Oxidative status
TAC
MDA liver
-0.21
0.67
Storage
Muscular lipids
0.25
Reproductive status
Gonadosomatic index
-0.33
Somatic condition
Hepatosomatic index
0.23
Body condition
-0.36
-0.26
Molecular
Individual
Biological level
B: Female
Physicochemical parameters
PCA axis 1 (T°C, -cond., -pH)
Pollutants
Pesticides
Pharmaceuticals
PCBs
PBDEs
PAHs
Organochlorines
Metals essential
Metals non-essential
0.26
0.37
-0.26
0.31
0.27
-0.28
0.39
-0.54
-0.60
Storage
Muscular lipids
0.40
Biotransformation
EROD
0.26
GST
Oxidative status
TAC
MDA gills
0.23
0.43
-0.47
0.61
-0.42
Histopathology
Liver
Gills
-0.49
Reproductive status
Gonadosomatic index
-0.35
Somatic condition
Hepatosomatic index
Body condition
-0.49
0.36
Molecular
Cellular
Individual
Biological level