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Effects of dietary and/or parental supplementation with selenium and mercury on their localisation in rainbow trout fry tissues (*Oncorhynchus mykiss*) by quantitative LA-ICP MS imaging

Laurie Labeyrie^a, Stéphanie Fontagné-Dicharry^b, Sandra Mounicou^{a,*}

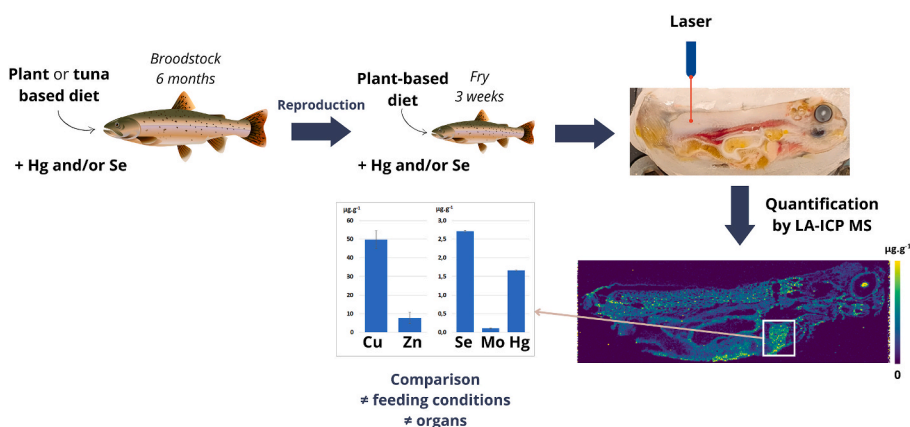
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HIGHLIGHTS

- Se intake enhanced hepatic Se level and decreased renal Hg level in fry.
- Dietary Se origin affected Se accumulation in liver, gut, kidney and flesh.
- Hg was weakly transferred to the progeny.
- Direct dietary Hg supplementation enhanced Hg accumulation in kidney.
- Colocalization of Hg and Se suggests a protective effect against Hg toxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing use of alternative feeds for sustainable aquaculture leads to a reduced selenium content requiring the use of supplements to maintain the antioxidant status of farmed fish. The introduction of tuna by-products in fish feed is attractive for their selenium content, but the presence of mercury limits their use as it may negatively impact fish health. Indeed, selenium and mercury metabolism, in relation to their interaction and biological effects in fish, is far from being fully understood. This study aims to assess the impact of parental and dietary supplementation with selenomethionine and methylmercury on selenium and mercury localisation and concentrations in tissues of 3-week rainbow trout fry. Six diets were used. Broodstock were fed a plant-based diet Bpc (with $0.2 \mu\text{g g}^{-1}$ selenium and $0 \mu\text{g g}^{-1}$ mercury) or a tuna by-product-based diet Bt (with $4 \mu\text{g g}^{-1}$ selenium and basal mercury at $0.2 \mu\text{g g}^{-1}$) during 6 months, supplemented or not with $4 \mu\text{g g}^{-1}$ of selenomethionine for the plant-based diet Bpo and/or with $2 \mu\text{g g}^{-1}$ of methylmercury for diets Bph and Bth. Their offspring were fed the plant-based diet Fpc, supplemented or not with the same levels of selenomethionine and methylmercury,

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until their third week. Mercury and selenium concentrations were determined in fry whole-body by ICP MS and quantitative images of selenium and mercury were produced by LA-ICP MS from fry whole-body thin sections. Dietary supplementation of fry with selenium and/or mercury increased their concentration in all tissues, especially in liver, kidney, muscle and intestine. Parental selenomethionine supplementation had a more pronounced effect on fry selenium levels than tuna by-products diet. Mercury transfer from broodstock to their offspring was weak. Mercury concentrations decreased in the kidney of fry supplemented with both selenium and mercury, which could be related to the presence of selenium.

1. Introduction

Reducing the use of fishmeal from dedicated industrial fisheries in aquafeeds is part of the solution to more sustainable aquaculture. Tuna by-products, which account for up to 70% of processed fish, could be an interesting alternative ingredient (The State of World Fisheries and Aquaculture 2022, 2022). However, tuna can contain contaminants such as mercury (Hg) at varying levels depending on the species, age and size (Kim et al., 2019; Milatou et al., 2020; Ormaza-González et al., 2020). Mercury has been identified by the World Health Organization as one of the ten chemical compounds of most concern to public health. In fish, Hg bioaccumulates as methylmercury (MeHg), which is one of the most toxic forms (Beckers and Rinklebe, 2017). Tuna may also contain high levels of selenium (Se) (Belmonte et al., 2021) known to protect living organisms from the toxicity of Hg (Amlund et al., 2015). An ongoing solution to replace fishmeal in aquaculture is the use of plant protein sources. However, due to their lower Se content compared to fishmeal, dietary Se supplementation is required to maintain normal biological functions and antioxidant system in fish (Fontagné-Dicharry et al., 2015; Wischhusen et al., 2019). The Se requirement for proper development of rainbow trout was determined to be between 0.35 and 0.65 $\mu\text{g g}^{-1}$ (Antony Jesu Prabhu et al., 2020; Hilton et al., 1980) but may be higher under stressful conditions (Kucukbay et al., 2006; Rider et al., 2009).

Different Se forms (organic and inorganic) and levels of supplementation were studied to assess their effects on the overall health of rainbow trout (Fontagné-Dicharry et al., 2015; Wischhusen et al., 2019). Organic Se (as hydroxy-selenomethionine (hydroxy-SeMet) or Se-enriched yeast) has been shown to be the most effective in maintaining the antioxidant system and increasing Se retention (Rider et al., 2009; Wischhusen et al., 2022). The effect of the form of supplementation on the potential transfer of Se from the broodstock to their progeny was also investigated. Wischhusen et al. (2022, 2020, 2019) found that Se accumulated in swim-up fry when their broodstock were supplemented, particularly with an organic Se form (as hydroxy-SeMet), and increased, for example, the mRNA level and activity of glutathione peroxidase 1 and the mRNA level of selenoprotein P, which are Se-dependent and involved in the antioxidant protection (Papp et al., 2007). Glutathione peroxidase 1 can metabolize hydrogen peroxide and organic hydroperoxides; selenoprotein P is involved in the transport of Se and can reduce phospholipid hydroperoxides. Different responses have been observed depending on the administered Se forms, and are therefore the result of different metabolism. Locating Se at the micro-metre scale in different organs and tissues of fish, can provide insights into the mechanisms by which Se is assimilated and metabolized.

Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP MS) is a key technique for elemental localisation, especially in small size biological samples, due to its spatial resolution (μm) and high sensitivity ($\mu\text{g.g}^{-1}$ and below) (Braeuer et al., 2022; Doble et al., 2021; Marković et al., 2021). It has been used by Wischhusen et al. (2021) to localise Se in swim-up and 11-week-fed fry receiving Se via parental or dietary supplementation with a plant-based diet. They reported that Se levels were influenced by parental diet in swim-up fry and by direct feeding in 11-week-fed fry. The yolk in swim-up fry and the liver, kidney and intestine of 11-week-fed fry were the most Se enriched organs. The organic form, hydroxy-SeMet, resulted in higher retention compared to sodium selenite, as well as higher Se signal intensities in muscle of

swim-up fry. Nevertheless, the inorganic form was more effective in increasing Se levels in 11-week-fed fry liver. However, due to analytical issues, only qualitative images were obtained and the determination of the concentrations would help to safely compare differences between organs and tissues depending on experimental conditions of feeding and supplementation.

The aim of the study was then to assess the effects of parental and dietary supplementation with organic Se and/or MeHg, with a plant-based or a tuna-based diet, on the localisation and concentration of Se, Hg and other trace elements (copper, zinc and molybdenum) in 3-week-fed rainbow trout fry. For that purpose, quantitative elemental images were produced by LA-ICP MS using two methods recently published (Labeyrie et al., 2023). Concentrations were determined for the first time in muscle, liver, kidney and intestine of 8 μm -thin and longitudinal sections of rainbow trout fry.

2. Material and methods

2.1. Experimental diets

All procedures to obtain rainbow trout samples were performed in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and the French Decree No. 2013-118 on animal experimentation. The feeding trial for broodstock and fry stages was approved by the C2EA-73 Ethics Committee and the French Ministry of Higher Education and Research (reference number APAFIS #32548-2021072309475577 v5) and was conducted at the INRAE experimental facilities at Léés-Athas and Donzacq (France, <https://doi.org/10.15454/GPYD-AM38>).

Rainbow trout broodstock with an initial mean body weight of 520 \pm 22 g were fed one of the six practical diets designed to differ in Se form and content and Hg levels (Table 1S1). The six diets based on plant-derived proteins only for diets Bpc, Bpo, Bph and Bpho or with 30% fishmeal derived from tuna by-products (Marine Biotechnology Products, Port-Louis, Mauritius) for diets Bt and Bth were formulated to contain 49% crude protein and 22% total lipid. The two plant-based diets Bpc and Bph were not supplemented with Se and had a basal Se level of 0.2 $\mu\text{g g}^{-1}$ diet. The broodstock diets Bpo and Bpho were supplemented with 4 $\mu\text{g Se.g}^{-1}$ diet as L-selenomethionine (Excellential Selenium 4000, Orffa, Breda, Netherlands) to a target total Se levels similar to the tuna-based diets Btc and Bth. Half of the diets (Bph, Bpho and Bth) were supplemented with 2 $\mu\text{g Hg.g}^{-1}$ diet as methylmercury(II) chloride (Sigma-Aldrich, Saint-Quentin-Fallavier, France). The diets were given for a period of 6 months prior to spawning. A beneficial effect of dietary SeMet supplementation and a detrimental effect of MeHg supplementation in plant-based diets was noticed on male growth (final mean weight: 761 \pm 34 vs. 581 \pm 36 g for SeMet-supplemented and non-SeMet-supplemented groups and 740 \pm 36 vs. 602 \pm 34 g for MeHg-supplemented and non-MeHg-supplemented groups, respectively) but not on female growth (827 \pm 268 g). At spawning, oocytes from each spawning female were collected by stripping, pooled, and fertilized with a pool of sperm obtained from males of the same dietary treatment collected on the same day. Eggs were cultured to swim-up fry stage. No significant dietary effect on reproductive performance was observed. At the swim-up fry stage (initial mean body weight: 87 \pm 8 mg for offspring from rainbow trout broodstock fed plant-based diets and 100 \pm 7 mg for

offspring from broodstock fed tuna-based diets), the pooled progeny of each parental treatment were divided into different fry feeding groups and fed one of the four fry plant-based diets (Fpc, Fph, Fpo, Fpho), which were designed to have similar Se and Hg levels compared to the broodstock diets for 3 weeks (Table 1SI). Surprisingly, Hg was found in the fry diet Fpo at $0.130 \mu\text{g g}^{-1}$. This was most likely due to a contamination during the preparation.

After 3 weeks of feeding with fry diets, fry (approximate size $3 \times 1 \text{ cm}$, final mean body weight: $331 \pm 29 \text{ mg}$ for offspring from rainbow trout broodstock fed plant-based diets and $380 \pm 33 \text{ mg}$ for offspring from broodstock fed tuna-based diets) were euthanized with an overdose of benzocaine, frozen in liquid nitrogen and stored at $-80 \text{ }^\circ\text{C}$. Similar to the growth performance of the male broodstock, a significant beneficial effect of SeMet supplementation in the plant-based broodstock diets was noticed for offspring growth ($343 \pm 21 \text{ mg}$ for SeMet-supplemented offspring vs. $320 \pm 31 \text{ mg}$ for control groups, $p = 0.004$), highlighting a parental effect. On the other hand, no significant effect of SeMet supplementation in the plant-based fry diet on fry growth was recorded.

2.2. Se and Hg total content analysis by ICP MS

Total Se and Hg contents in rainbow trout diets, eggs and 3-weeks whole-body fry were determined by ICP MS by Ultra Trace Analysis Aquitaine (UT2A, Pau, France) using the following protocol. An aliquot of 0.5–1 g of fresh sample was digested in 2 mL of ultrapure HNO_3 (70% v/v, ULTRATRACE PPB GRADE, Scharlau, Atlantic Labo, Bruges, Belgium) and 5 mL of HCl (35% v/v, Sigma-Aldrich, Saint-Quentin-Fallavier, France) in a controlled heating block (DigiPrep, SCP Science) during 3 h at $85 \text{ }^\circ\text{C}$. Then the digest was filled up to 50 mL as final volume with ultrapure water (Merck MilliQ system, Millipore, Molsheim, France). Total Se and Hg were quantified by Inductively Coupled Plasma Mass Spectrometry (7800 ICP MS, Agilent Technologies, Tokyo, Japan), measuring ^{77}Se , and ^{78}Se , ^{200}Hg and ^{202}Hg isotopes, with activation of the collision cell technology using dihydrogen (H_2) as gas to remove polyatomic interferences. For quantification, the calibration was performed by standard addition of Se and Hg (from 0.1 to $50 \mu\text{g.L}^{-1}$) in a set of digested samples. Quality control solutions were prepared by spiking a set of digested samples with Se and Hg at $5 \mu\text{g.L}^{-1}$ which were then regularly analyzed within the samples sequence. In addition, DOLT-5, TORT-3 and ERM-464 reference materials with certified values for Se (except ERM-464) and Hg were used for methodology validation. Means $[\pm \text{standard deviation (SD)}]$ Se recoveries were of $96.1 \pm 0.2 \%$, $94 \pm 0.2 \%$ for DOLT-5 and TORT-3 respectively. For Hg, recoveries were of $100.47 \pm 0.02 \%$, $97.70 \pm 0.01 \%$, $97.7 \pm 0.1 \%$, for DOLT-5, TORT-3 and ERM-464, respectively. Three sample replicates for each feeding condition were analyzed. The LOQ of the UT2A method was $0.015 \mu\text{g g}^{-1}$ for Hg and $0.020 \mu\text{g g}^{-1}$ for Se.

2.3. LA-ICP MS instrumentation and parameters

For LA-ICP MS analysis, an Agilent 7700x model (Agilent Technologies, Tokyo, Japan) equipped with Ni cones and a Micromist nebulizer (for initial instrument tuning) was used. The detection parameters were optimized and the mass calibration was checked using a solution of $1 \mu\text{g.L}^{-1}$ of Li, Y, Ba, Tl and Ce in 2% HNO_3 . The detector was then coupled to a laser ablation system (NWR 213, ESI, Fremont, CA, USA). Helium was used at 800 mL min^{-1} to transport the ablated material through a TV2 ablation cell and mixed with argon prior to introduction into the ICP dry plasma. Dihydrogen (H_2) was used as collision gas (between 3.8 and 4 mL min^{-1}) to remove interferences. The detection parameters (torch position, lenses) were then adjusted by ablation of a NIST 612 glass standard (NIST, Washington, USA).

2.4. LA-ICP MS quantitative imaging

2.4.1. Reagents

For the LA-ICP MS quantitative method, dextran polymer (approximate $\text{Mw } 500\,000 \text{ g mol}^{-1}$) was purchased from VWR (Fontenay-sous-Bois, France) and elemental solutions at $50\,000 \text{ mg.L}^{-1}$ (Cu and Zn), $10\,000 \text{ mg.L}^{-1}$ (Se, Mo and Hg) and 1000 mg.L^{-1} (Ge and Te, used as internal standards) were purchased from SCP Science (PlasmaCAL, Courtaboeuf, France). Intermediate solutions were prepared in 2% ultrapure HNO_3 (69–70% ULTRATRACE PPB GRADE, Scharlau, Atlantic Labo, Bruges, Belgium). All solutions were prepared with Milli-Q water (resistivity $\geq 18.2 \Omega \text{ cm}$) obtained from a Milli-Q Elix water purification system (Millipore, Molsheim, France).

2.4.2. Sample preparation

For LA-ICP MS analysis, rainbow trout fry thin sections were prepared by cryomicrotomy (CM, 1950 model, Leica, France) with the chamber and sample holder temperatures set to $-15 \text{ }^\circ\text{C}$ and $-18 \text{ }^\circ\text{C}$, respectively. The fishtail (considered as an uninteresting part of the fish) of the fry was cut to fit the specimen disc before direct embedding of the fry in water. Sections of $8 \mu\text{m}$ were produced, in which we could identify all organs and tissues, and deposited on polymer films substrate containing the internal standards for signal normalization and further quantification. The sections were then placed in boxes and stored until their analysis, maximum 3 days after their cutting.

2.4.3. Laser ablation and ICP MS parameters

The integration time was set to 0.08 s for ^{63}Cu , ^{64}Zn and ^{202}Hg , 0.1 s for ^{78}Se , ^{80}Se and ^{98}Mo , and 0.05 s for ^{126}Te and ^{72}Ge , resulting in a total sampling time of 0.658 s. The same laser parameters were used for standards and rainbow trout fry: $60 \times 60 \mu\text{m}$ laser spot, line scan speed of $90 \mu\text{m s}^{-1}$ and laser shot repetition rate of 20 Hz. For rainbow trout fry imaging, a distance of $60 \mu\text{m}$ was used between each ablated line. The laser fluency was adjusted to approximately 4.5 J cm^{-2} to enable the ablation of the entire thickness of the sample section.

2.4.4. Quantification methods: polymer films and muscle standards

The methods used for Se, Hg, Cu, Zn and Mo quantitative imaging in rainbow trout fry have been recently published by our group (Labeyrie et al., 2023). In brief, two types of calibration standards for LA-ICP MS were produced, i.e. polymer films standards and matrix-matched standards (based on trout muscle or calf liver), both spiked at different levels of Se, Hg, Cu, Zn and Mo. Elemental concentrations were determined by ICP MS total analysis in each produced standard. Matrix-matched standards were used to validate polymer films quantification method which provided good agreement with ICP MS concentration values (i.e. 1% and 16% difference for Se in $2.5 \mu\text{g g}^{-1}$ and $40 \mu\text{g g}^{-1}$ trout muscle standard). Only matrix matched standards methodology provided linear regression for Hg quantitative LA-ICP MS analysis. The two types of matrices were used to determine Hg concentrations in fry tissues and a 25% difference of Hg concentrations could be assessed. For Cu, Se and Mo quantification, the polymer film standardisation method was used. ^{78}Se signals were normalized to ^{126}Te signals, whereas ^{63}Cu and ^{98}Mo signals were normalized to ^{72}Ge . For Hg quantification, homogenized and spiked trout muscle standards were used and their signals normalized to ^{72}Ge signals. To generate calibration curves, 2–3 scans per film and 3–4 scans per muscle standard section were analyzed and normalized intensities were plotted as a function of the standard concentrations determined by ICP MS, as described by Labeyrie et al. (2023). The limits of detection (LODs) and the limits of quantification (LOQs) were 1.6, 5.3, 0.16, 0.09, $0.3 \mu\text{g g}^{-1}$ and 5.2, 18, 0.55, 0.32, $1.0 \mu\text{g g}^{-1}$ for Cu, Zn, Se, Mo, Hg respectively.

2.4.5. Quantitative images production

An ICP MS acquisition file (csv format) was obtained for each scanned line with LA. For the imaging experiment, a custom Python program

was used to generate an image representing element concentrations per pixel with a colour code after blank subtraction and calibration. For precise quantification in organs and tissues, areas were delimited on the images and normalized intensities were averaged. Concentrations presented in this work are the mean of biological triplicates.

2.5. Statistical analysis

Data are expressed as mean \pm their standard deviation. Differences between groups were analyzed by one-way ANOVA. Where needed, a Tukey-Kramer's post hoc test was used to determine differences between groups. Differences were considered significant if $p < 0.005$.

3. Results and discussion

3.1. Selenium and mercury content in whole-body rainbow trout fry

Selenium and Hg concentrations in the different rainbow trout fry whole-body were determined by ICP MS and presented in Table 1.

As expected, no Hg was found in the fry fed the control diet (Bpc-Fpc, $< \text{LOQ}$). Hg was present at a similar concentration (between 0.58 and $0.59 \mu\text{g g}^{-1}$) in the fry directly supplemented (Bpc-Fph, Bpc-Fpho and Bph-Fph) with a plant-based Hg diet. No increase in Hg levels could be detected in fry from broodstock fed MeHg-supplemented diets. As expected from its quantification in the diet (Table 1SI), Hg was found at $0.048 \pm 0.007 \mu\text{g g}^{-1}$ (average) in the different offspring groups fed the Fpo diet (including groups not shown here, Bpc-Fpo ($0.043 \mu\text{g g}^{-1}$), Bph-Fpo ($0.057 \mu\text{g g}^{-1}$), Bpho-Fpo ($0.049 \mu\text{g g}^{-1}$), Bth-Fpo ($0.054 \mu\text{g g}^{-1}$)). However, it was absent in Bpho-Fpc and Bth-Fpc fry, but was found at $0.024 \pm 0.009 \mu\text{g g}^{-1}$ ($0.092 \pm 0.006 \mu\text{g Se.g}^{-1}$) in Bph-Fpc fry (data not shown), whose supplementation was only parental possibly suggesting a greater transfer of Hg to fry with lower Se levels.

Mercury was also quantified in eggs (mean weight: $62 \pm 11 \text{ mg}$) by ICP MS (Table 2SI) and was determined to be $0.185 \pm 0.034 \mu\text{g g}^{-1}$, $0.087 \pm 0.002 \mu\text{g g}^{-1}$ and $0.059 \pm 0.019 \mu\text{g g}^{-1}$ in eggs coming from broodstocks Bph, Bpho and Bth, respectively (no significant differences between the last two). These values were low, representing only 3–8% of the Hg content in the diet, indicating the low transfer of this element from the broodstock to their progeny. Interestingly, compared to broodstock supplementation with Hg only, less than half of the Hg was transferred to the eggs when the broodstock were fed diets containing both Se and Hg, compared to supplementation on the broodstock with Hg alone. Furthermore, after 3 weeks of feeding, no Hg could be detected in Bpho-Fpc and Bth-Fpc fry, in contrast to Bph-Fpc fry, although their Hg concentration was reduced by more than 7 compared to the concentration at the egg stage. This could have several

Table 1

Selenium and mercury concentrations in whole-body of 3-week rainbow trout fry, results expressed as fresh weight.

Fry diet condition	Fry mean body weight, mg	Se, $\mu\text{g.g}^{-1}$ in fry	Hg, $\mu\text{g.g}^{-1}$ in fry
Bpc-Fpc	$300 \pm 10^{\text{cd}}$	$0.087 \pm 0.015^{\text{a}}$	$< \text{LOQ}$
Bpc-Fph	$287 \pm 23^{\text{d}}$	$0.101 \pm 0.013^{\text{a}}$	$0.579 \pm 0.011^{\text{a}}$
Bph-Fph	$334 \pm 8^{\text{bcd}}$	$0.094 \pm 0.005^{\text{a}}$	$0.577 \pm 0.080^{\text{a}}$
Bpc-Fpho	$315 \pm 23^{\text{cd}}$	$1.10 \pm 0.10^{\text{e}}$	$0.589 \pm 0.060^{\text{a}}$
Bpho-Fpc	$376 \pm 14^{\text{ab}}$	$0.374 \pm 0.035^{\text{b}}$	$< \text{LOQ}$
Bpo-Fpo	$343 \pm 19^{\text{bc}}$	$1.58 \pm 0.02^{\text{e}}$	$0.042 \pm 0.001^{\text{b}}$
Bth-Fpc	$407 \pm 16^{\text{a}}$	$0.121 \pm 0.010^{\text{d}}$	$< \text{LOQ}$
Bt-Fpo	$329 \pm 2^{\text{bcd}}$	$1.28 \pm 0.07^{\text{e}}$	$0.047 \pm 0.006^{\text{b}}$

For each column, means not sharing a common superscript letter are significantly different according to one-way ANOVA followed by Tukey's post hoc test.

explanations. The higher Hg concentration in Bph eggs and its persistence in 3-week-fed Bph-Fpc fry could be due to the higher Hg concentration in the Bph broodstock diet compared to the others ($2.21 \mu\text{g g}^{-1}$ for Bph, $1.49 \mu\text{g g}^{-1}$ for Bpho and $1.85 \mu\text{g g}^{-1}$ for Bth). It could also be explained by the higher Se concentration in Bpho and Bth broodstock diets, with 4.95 and $3.7 \mu\text{g g}^{-1}$, respectively, which reduces the transfer of Hg to the progeny. The decrease in Hg concentration between egg stage and fry after 3 weeks of feeding may then be either natural or enhanced by the presence of Se in these fry.

Not surprisingly, the lowest Se concentration was found in the fry receiving the control diet Bpc-Fpc ($0.087 \pm 0.015 \mu\text{g g}^{-1}$). Similar concentrations have been reported in other studies in control fry receiving a plant-based diet (about 0.12 – $0.2 \mu\text{g g}^{-1}$ with $0.3 \mu\text{g g}^{-1}$ Se in the diet) (Bidon et al., 2023; Wischhusen et al., 2019, 2021, 2022).

Selenium concentrations in Bpc-Fph and Bph-Fph fry were slightly higher (16 and 8% increase, respectively) but not significantly different from the control fry. Similarly, no significant differences were found between eggs coming from Bpc ($0.213 \pm 0.006 \mu\text{g g}^{-1}$) and from Bph ($0.248 \pm 0.014 \mu\text{g g}^{-1}$) broodstock (Table 2SI). As expected, the Se concentration was higher in the Bpc-Fpho, Bt-Fpo and Bpo-Fpo fry fed diets supplemented with SeMet, suggesting that direct feeding is the most efficient uptake. Since the Se supplementation since the broodstock feeding (Bt-Fpo and Bpo-Fpo) increased the Se level in the fry between 14 and 30% (compared to Bpc-Fpho) and that the Bpho-Fpc diet also significantly increased the Se concentration ($0.374 \mu\text{g g}^{-1}$) compared to the control, we may assume that the Se supplementation of the broodstock also helps in Se assimilation and transfer to the progeny. In addition, and in contrast to Hg, this is supported by the higher Se transfer in the eggs. Indeed, Se concentrations in eggs of Se-supplemented broodstock were $4.25 \pm 0.39 \mu\text{g g}^{-1}$ and $4.37 \pm 0.15 \mu\text{g g}^{-1}$ for Bpo and Bpho conditions and $0.213 \pm 0.006 \mu\text{g g}^{-1}$ and $0.248 \pm 0.014 \mu\text{g g}^{-1}$ for Bpc and Bph conditions, respectively. This was a 19-fold increase compared to the Bpc and Bph control conditions, close to the difference in dietary Se, with a 24-fold higher Se content in the Bpo and Bpho diets than in the Bpc and Bph diets. This was also observed by Wischhusen et al. (2021, 2019) with higher Se levels in swim-up fry when the broodstock was Se-supplemented compared to the control diet. It can therefore be concluded that broodstock can transfer high Se levels to their progeny.

We could also notice that the concentration of Se in the fry was higher when the broodstock received Se via a plant-based diet supplemented with SeMet (Bpho-Fpc) than when it came from tuna meal (Bth-Fpc), with quite similar elevated levels of Se in the diets ($5.0 \mu\text{g g}^{-1}$ for Bpho and $3.7 \mu\text{g g}^{-1}$ for Bth). This was also shown by the Se concentration in eggs, with 80% higher values for Bpo and Bpho compared to Bt and Bth. Bidon et al. (2023) also measured slightly higher Se concentrations in 3-week-fed rainbow trout juveniles directly with a plant-based diet supplemented with SeMet than in fry fed a tuna-based diet, although the Se burden of the supplemented plant diet ($2 \mu\text{g g}^{-1}$) was significantly lower than the Se content of the tuna-based diet ($7.2 \mu\text{g g}^{-1}$). In the same study, MeHg was also present in both diets (plant and tuna based diets; 1.5 and $1.9 \mu\text{g g}^{-1}$ respectively). We could have interpreted this difference in Se concentration as the presence of Hg, but Bidon et al. (2023) found similar Se concentrations in the whole body of rainbow trout juveniles fed the plant-based diets supplemented with SeMet with and without the presence of MeHg, and in juveniles fed the tuna-based diets in the presence or absence of MeHg, confirming the absence of effect of MeHg on Se levels under these conditions. This difference in Se concentration in fry may be due to the higher availability of Se from SeMet than from fishmeal as reported by Watanabe et al. (1997). Similarly, in the present study, the Bpo-Fpo fry were more concentrated in Se than the Bt-Fpo fry, also showing the influence of the origin of the broodstock Se supplementation.

3.2. Effects of diets on selenium localisation in rainbow trout fry

Quantitative images of ⁷⁸Se for one replicate of each dietary condition are presented in Fig. 1, the other two are presented in Fig. 1SI in the supplementary information. The major organs and tissues (muscle, kidney, intestine, liver, brain, eye) of rainbow trout fry were identified by comparison with images taken before cryomicrotomy (right in

Fig. 1). In five individuals, the kidney was absent from the section or difficult to find on the ⁷⁸Se mapping, possibly due to difficulties in cryomicrotomy sectioning. From the LA-ICP MS images (Fig. 1, left side), we could see that Se concentrations were close to the quantification limit in fry not supplemented with SeMet (Bpc-Fpc < Bpc-Fph ~ Bph-Fph < Bth-Fpc), with the morphology of the fry barely distinguishable. Selenium concentrations were clearly determined in the

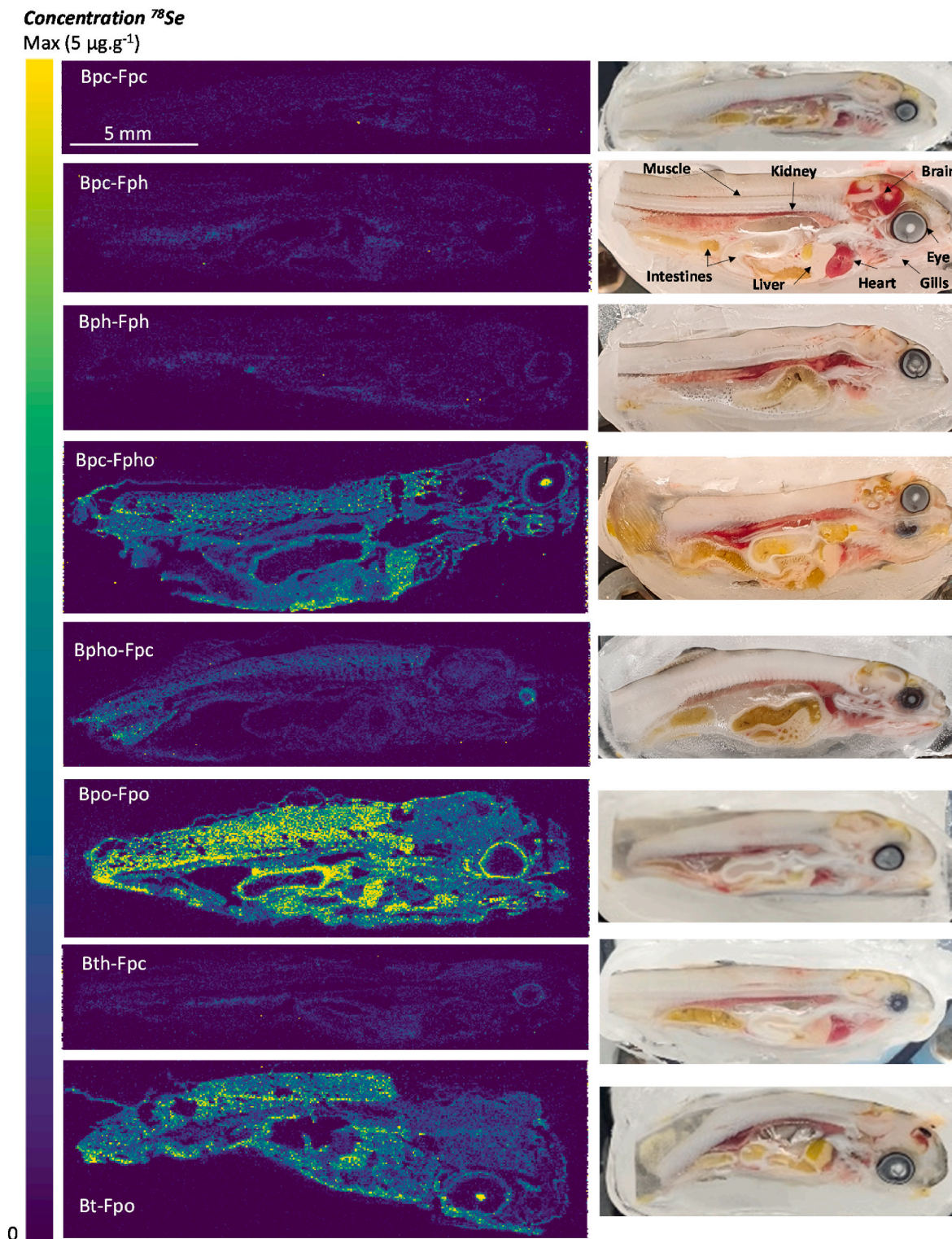


Fig. 1. Quantitative images of ⁷⁸Se in thin sections (8 µm) of entire 3-week rainbow trout fry under various feeding conditions (left), alongside their corresponding images of the embedded fry just before cutting (right).

whole body of rainbow trout fry with the highest levels in fry fed diets supplemented with SeMet (Bpo-Fpo > Bt-Fpo > Bpc-Fpho) whereas Se concentrations in the Se-supplemented broodstock map (Bpho-Fpc) were much weaker. This visual trend of Se concentrations in LA-ICP MS images is consistent with the total content trend determined by ICP MS for whole fry (Table 1). In addition, a refined image analysis was performed and Se concentrations in muscle, liver, kidney and intestine of the rainbow trout fry fed the 8 different diets were determined by integrating areas on the quantitative images. The average of Se concentrations in all fry organs determined by LA-ICP MS were compared with the ICP MS total analysis data (Fig. 2) and confirmed the observed trend.

The concentrations determined by LA-ICP MS were higher than those obtained via ICP MS. This disparity may be attributed to the dehydration of the fry sections during LA-ICP MS analysis caused by the helium gas flow within the ablation cell and to the use of fresh sample (whole body) for ICP MS total analysis. It could also be explained by the presence of other biological materials low in Se in whole-body fry, thereby diluting the overall concentration of this element during the total content analysis conducted by ICP MS. However, the trends in concentration variations determined by both techniques across different diets were consistent.

In the control fry (Bpc-Fpc), although Se was detected, it was below the LOQ and estimated concentrations were similar in all organs, except for muscle where it was slightly lower.

In fry exposed to MeHg, either through direct feeding or parental feeding (Bpc-Fph and Bph-Fph), the estimated Se concentrations were between the LOD and the LOQ, except for the kidney of Bpc-Fph fry where the estimated Se concentrations were slightly above the LOQ. A similar trend was observed in Bth-Fpc fry, although to a lesser extent. Reduced scale images are presented in Fig. 2SI to illustrate this observation.

As expected from the pictures, direct dietary supplementation of fry with SeMet increased Se concentrations in all organs and tissues of the Bpc-Fpho, Bpo-Fpo and Bt-Fpo fry, similar to what was observed in previous studies in rainbow trout (Fontagné-Dicharry et al., 2015; Godin

et al., 2015; Wischhusen et al., 2021). Muscle, intestine, liver and kidney were Se enriched compared to the other organs and tissues. Se levels were also high in the eye and its periphery and in the brain, but to a lesser extent. All these organs have already been reported to accumulate Se in salmonids (Hilton et al., 1982; Misra et al., 2012; Pacitti et al., 2015; Sele et al., 2018; Wischhusen et al., 2021). For these feeding conditions (Bpc-Fpho, Bpo-Fpo and Bt-Fpo), the liver was the organ most enriched in Se (2.61, 4.14 and 4.63 $\mu\text{g g}^{-1}$ respectively), followed by the intestine and the muscle (1.96, 2.64 and 3.28 $\mu\text{g g}^{-1}$ for the intestine and 1.67, 3.09 and 2.60 $\mu\text{g g}^{-1}$ for the muscle), then the kidney (1.49, 2.39 and 1.41 $\mu\text{g g}^{-1}$). It should also be noted that the Se concentration in the muscle and the kidney was the highest in the Bpo-Fpo condition, while the liver and intestine showed the highest concentrations in the Bt-Fpo condition. Wischhusen et al. (2021) also reported liver and intestine as organs with high Se intensity. However, in contrast to the present study, Se was less abundant in the muscle than in the kidney and the intestine, but they used lower concentrations of SeMet supplementation (0.6 $\mu\text{g Se.g}^{-1}$ for the highest concentration). Similarly, Misra et al. (2012) found a different order of Se levels for rainbow trout fed a higher concentration of SeMet (40 $\mu\text{g Se.g}^{-1}$): with stomach > brain > liver > kidney > muscle. This may indicate the influence of the level of Se supplementation and of different feeding conditions of the broodstock and fry, which may affect the assimilation, metabolism and excretion of Se and thus its levels in different tissues. This was already evidenced by Hilton et al. (1982) who found different Se proportions in liver and kidney of trout depending on Se diet level. They mentioned that the highest concentrations of Se occurring in the liver and kidney, could reflect possible detoxification and excretory roles for these tissues. They also reported that a fixed Se amount was defecated and that the excess of Se was excreted by the kidney. Similar mechanism might also take place in our study.

When the Se supplementation was only parental and in the presence of Hg (Bpho-Fpc and Bth-Fpc), Se was detected in all tissues of the fry. Contrary to the direct dietary supplementation, the liver was no longer the most concentrated organ, and the concentrations were quite similar in all organs except for the muscle of Bth-Fpc fry, which had the lowest

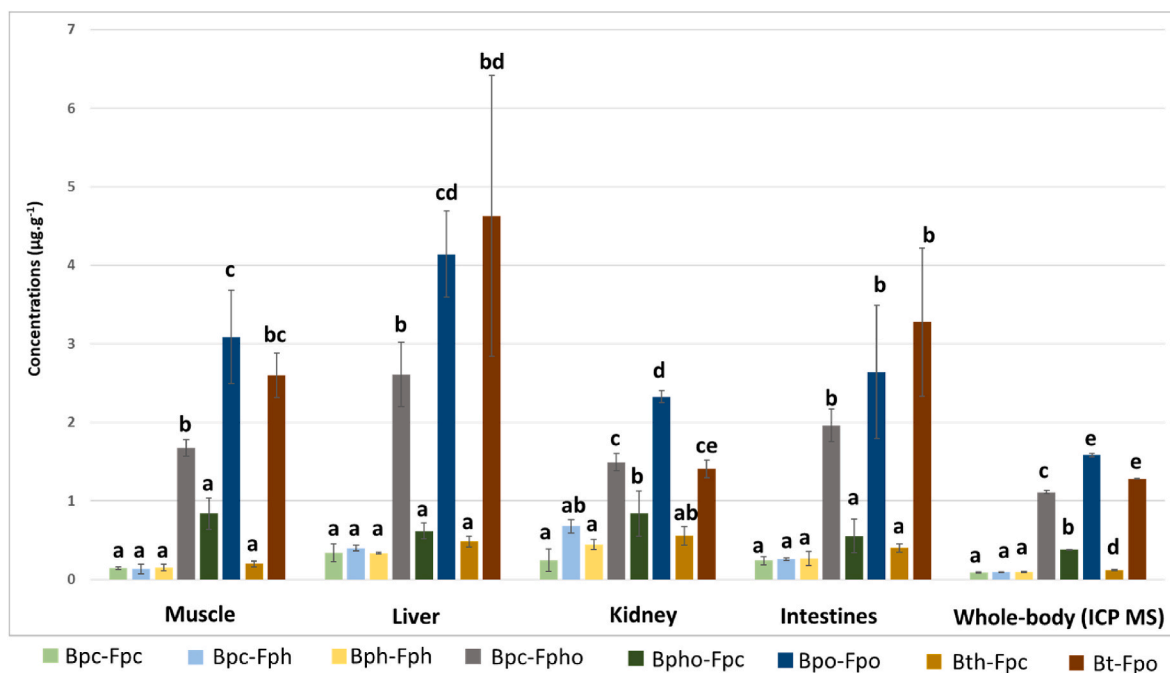


Fig. 2. Quantification of ^{78}Se by LA-ICP MS (triplicate mean) in organs of fry from different treatments (Bpc-Fpc in light green, Bpc-Fph in light blue, Bph-Fph in light yellow, Bpc-Fpho in grey, Bpho-Fpc in dark green, Bpo-Fpo in dark blue, Bth-Fpc in dark yellow and Bt-Fpo in brown) and comparison with the Se concentrations determined in whole body of fry (fresh sample) by ICP MS. Differences between fry conditions were assessed for each organ. For each organ, means not sharing a common superscript letter are significantly different according to one-way ANOVA followed by Tukey-Kramer's test.

Se content ($0.84 \mu\text{g g}^{-1}$ for muscle and kidney, 0.61 for liver and $0.55 \mu\text{g g}^{-1}$ for the intestine of Bpho-Fpc; concentration between LOD and LOQ for Bth-Fpc). Wischhusen et al. (2021) detected Se in swim-up fry from broodstock supplemented with hydroxy-SeMet but not in 11-week-fed fry. Dietary supplementation of broodstock still seems to have an effect on Se levels in 3-week-fed fry where parental Se ranged from 50% (calculated as absolute quantity of Se) of whole-body fry Se in the Bpo-Fpo group to 191% in the Bpho-Fpc group, but no longer in

11-week-fed fry. However, it should be noted that their supplementation level ($0.7 \mu\text{g g}^{-1}$) was lower than in the present study ($5 \mu\text{g g}^{-1}$), which may explain the persistence of Se in organs in our study and its absence in the 11-week-fed fry.

LA-ICP MS imaging also showed that Se supplementation of both the broodstock and the fry (Bpo-Fpo and Bt-Fpo) increased the Se concentration in all organs of the fry, except the kidney, compared to fry dietary supplementation only (Bpc-Fpho).

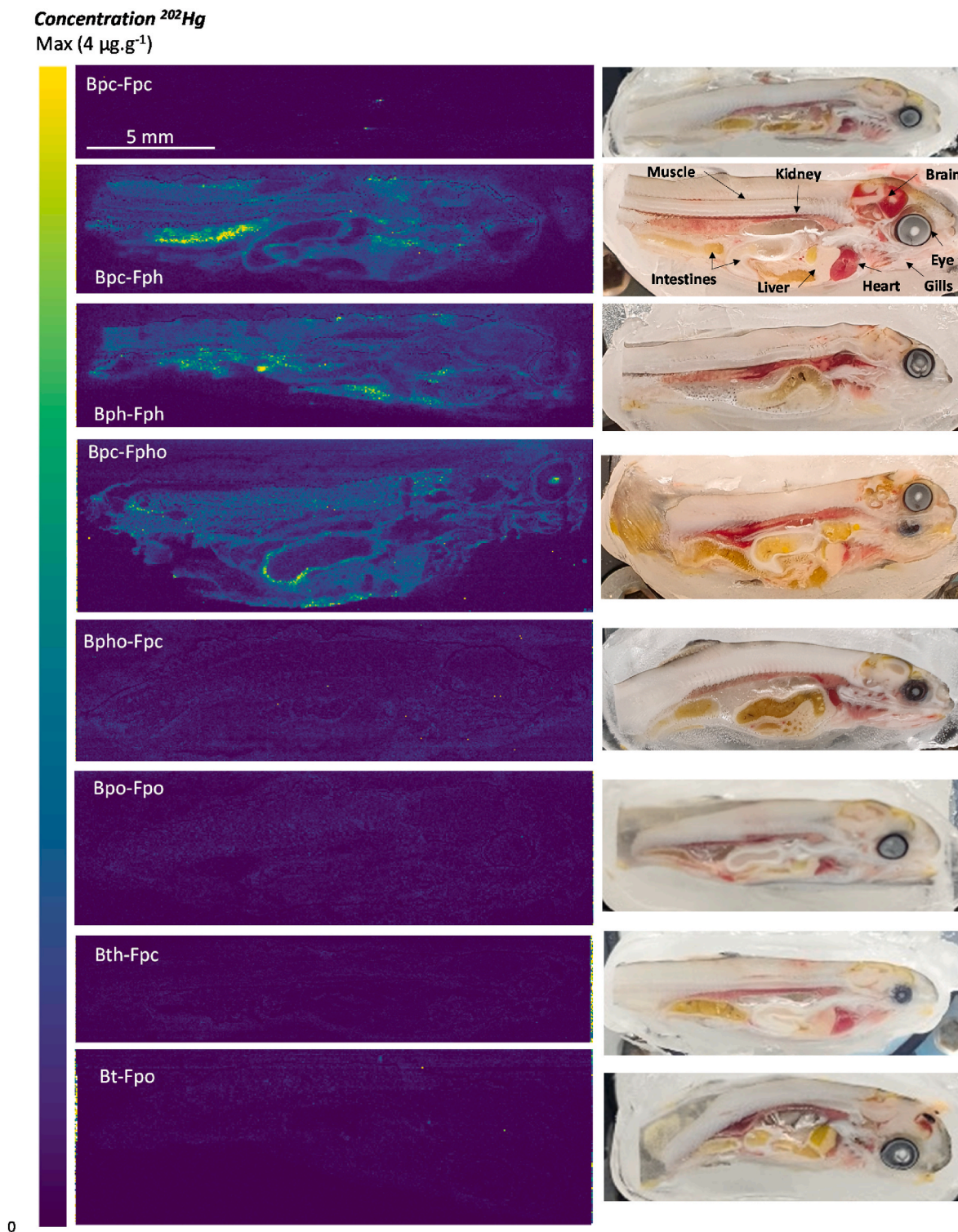


Fig. 3. Quantitative images of ^{202}Hg in thin sections ($8 \mu\text{m}$) of whole 3-week-fed rainbow trout fry from different feeding conditions (left) and their images before cryomicrotomy (right).

As seen in paragraph 3.1., the whole-body concentration of Se in Bt-Fpo determined by ICP MS was lower than that of Bpo-Fpo. However, the mean Se concentrations in all organs determined by LA-ICP MS were not significantly different (Fig. 3SI). In addition, as shown in Fig. 2, the mean concentration in the liver of Bt-Fpo fry determined by LA-ICP MS was higher than that of Bpo-Fpo but with a significant error. This was due to one replicate being higher than the other two. Without the elevated concentration of this replicate, the mean Se concentration in Bt-Fpo liver was $3.5 \pm 0.5 \mu\text{g g}^{-1}$ which was lower than Bpo-Fpo ($4.1 \pm 0.6 \mu\text{g g}^{-1}$), although not significantly different. The same was observed for the intestine, with different Se concentrations for the three replicates, making it difficult to draw firm conclusions. Nevertheless, considering the important but equal errors in Se concentrations in the gut of Bpo-Fpo and Bt-Fpo fry, Se concentration tended to increase from Bpo-Fpo to Bt-Fpo. This may indicate that Se from tuna-based diets is less bioavailable than Se from plant-based diets. The only organ that was significantly different between Bpo-Fpo and Bt-Fpo was the kidney. This may be explained by the difference in the broodstock diet, with Se from tuna being less assimilated and less metabolized in the kidney compared to a SeMet-supplemented plant-based diet.

3.3. Effects of diets on mercury localisation in rainbow trout fry

As expected, no Hg was found in the control Bpc-Fpc fry (Fig. 3). From the quantitative images, it can be seen that direct dietary MeHg supplementation in fry increased Hg level in the whole body of the fry (Bpc-Fph, Bph-Fph and Bpc-Fpho). This has already been reported by Bidon et al. (2023) for 3-week-fed rainbow trout juvenile fed a plant-based diet supplemented with MeHg. The same was observed for the other two replicates for each condition (Fig. 4SI). As for Se, LA-ICP MS allowed the quantification of Hg in each organ (Fig. 4). In Bpc-Fph, Bph-Fph and Bpc-Fpho fry, all Hg concentrations were above the LOD (estimated values are given), but not always above the LOQ. The concentration in the kidney of Bpc-Fph fry was higher and significantly different from the other organs of the same condition and from the organs of the other fry, except for the kidney of Bph-Fph fry. The Hg concentration in the kidney of Bph-Fph fry was not significantly

different from the other organs of the same feeding condition or from the organs of Bpc-Fpho fry, but seemed to be slightly higher. It has already been reported that the kidney preferentially accumulates Hg in wild fish (Belmonte et al., 2021) and in fish fed a MeHg-supplemented diet (Raihan et al., 2020). Nevertheless, the distribution of Hg between organs seems to be different in Bpc-Fpho, which exhibits similar concentrations for all organs (around $1.5 \mu\text{g g}^{-1}$) compared to Bpc-Fph and Bph-Fph. This observation could be related to the presence of SeMet in the diet of the fry, which affects Hg assimilation and thus reduces the concentration of Hg in the kidney.

As explained in 2.1 and shown in Table 1SI, the Fpo diet was contaminated with Hg. Mercury was detected in all the replicates of Bpo-Fpo and Bt-Fpo, but it was not possible to estimate the Hg concentration as it was slightly above the background level. Qualitative images (and reduced scale intensity) are presented in the Supplementary Information (Fig. 5SI), showing the presence of Hg in muscle and intestine, but not in liver and kidney.

In addition to Hg quantification in organs, Hg was also found in the brain of the rainbow trout fry at less than $1 \mu\text{g g}^{-1}$, as shown in the Bpc-Fph, Bph-Fph and Bpc-Fpho images in Fig. 3. Mercury has also been quantified by ICP MS or atomic absorption in the brains of MeHg-supplemented zebrafish (Amlund et al., 2015) and Atlantic salmon (Berntssen et al., 2003), as well as in wild Atlantic bluefin tuna (Belmonte et al., 2021). In zebrafish fed diets supplemented with $5 \mu\text{g Hg g}^{-1}$ for 8 weeks, Hg accumulated more in the brain ($22 \pm 7 \mu\text{g g}^{-1}$ ww) as well as in the liver ($14 \pm 4 \mu\text{g g}^{-1}$ ww), compared to the muscle ($3.4 \pm 0.2 \mu\text{g g}^{-1}$ ww). However, in Atlantic salmon, the Hg concentration was lowest in the brain ($1.2 \pm 0.3 \mu\text{g g}^{-1}$ ww) compared to the liver ($4.1 \pm 1.1 \mu\text{g g}^{-1}$ ww) and the kidney ($2.0 \pm 0.2 \mu\text{g g}^{-1}$ ww) after 4 months of feeding diets containing $5 \mu\text{g Hg g}^{-1}$ as MeHg. Similarly, in wild tuna, the brain showed the lowest accumulation levels compared to the other organs (kidney > muscle > liver > gills > bones > brain, with 4.8 ± 0.4 , 0.7 ± 0.0 , 0.6 ± 0.0 , 0.5 ± 0.0 , 0.3 ± 0.0 and $0.2 \pm 0.0 \mu\text{g g}^{-1}$ ww, respectively). It shows that Hg accumulation in the different organs differs between fish species. The levels found in our samples (Bpc-Fph, Bph-Fph and Bpc-Fpho) were more similar to those quantified in salmon and tuna than in zebrafish.

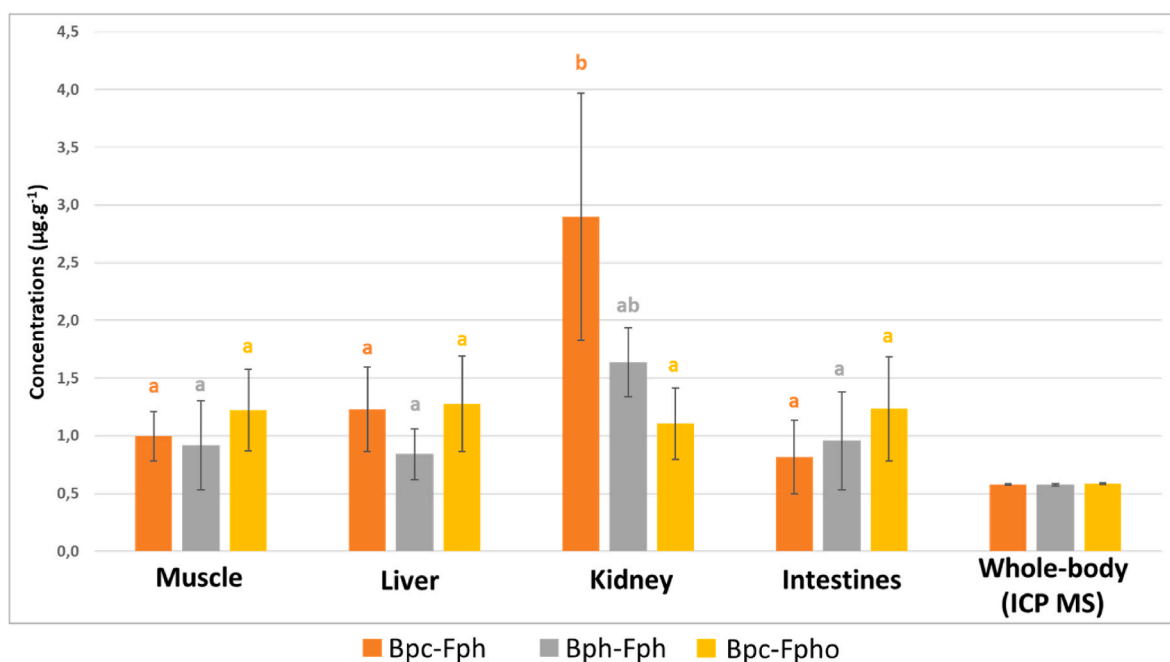


Fig. 4. Quantification of ^{202}Hg by LA-ICP MS (triplicate mean) in organs of fry of different treatments (Bpc-Fph in orange, Bph-Fph in grey and Bpc-Fpho in yellow) and comparison with concentrations determined in the whole-body of fry by ICP MS. For each organ, means not sharing a common superscript letter are significantly different according to one-way ANOVA followed by Tukey-Kramer's test.

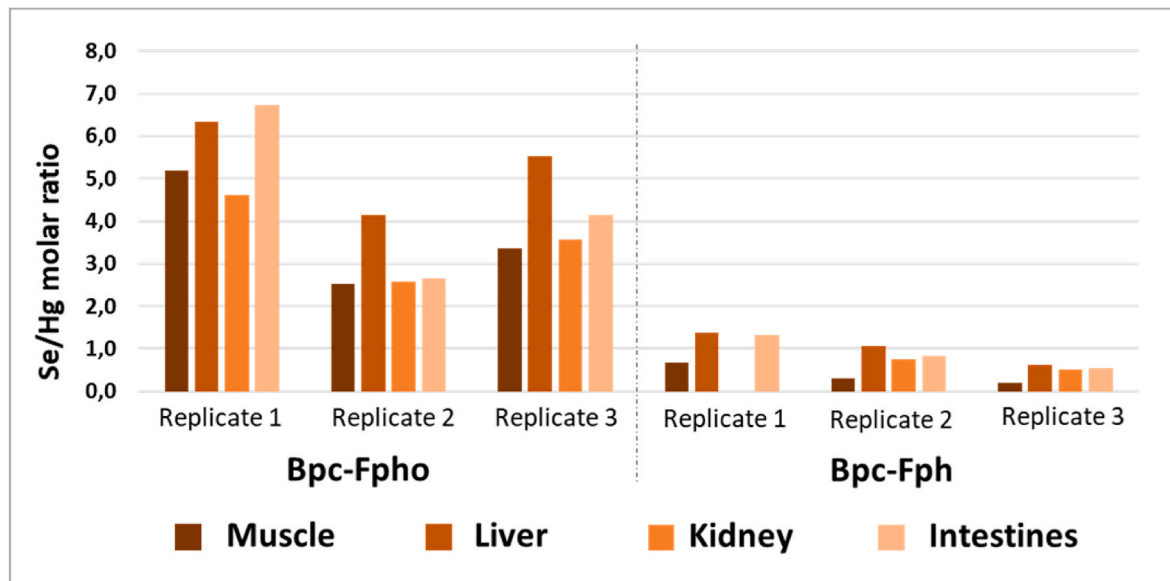


Fig. 5. Se/Hg molar ratio in each organ or tissue of Bpc-Fpho (left) and Bpc-Fph (right). Kidney could not be identified in replicate 1 of Bpc-Fph.

As discussed for ^{78}Se , the concentrations determined for ^{202}Hg by ICP MS were lower than those determined by LA-ICP MS due to dilution caused by the fact that fresh sample is used for total ICPMS analysis and by the presence of other fry matrix tissues poor in Hg.

3.4. Effects of mercury presence in diet on selenium localisation in rainbow trout fry

In Bpc-Fpho fry where both MeHg and SeMet were added directly to the fry diet, Se and Hg colocalized in whole body of the fry. Se/Hg molar ratios were calculated as this is a good indicator of the protective effect ($\text{Se}/\text{Hg} > 1$) of Se over Hg deleterious effects (Ralston et al., 2008). These molar ratios were higher in all organs of Bpc-Fpho than in Bpc-Fph fry (Fig. 5). In Bpc-Fpho fry, they ranged from 2 (replicate 2) to 6.7 (replicate 1), reflecting a protective effect of Se against Hg toxicity. In Bpc-Fph fry, however, they were mostly below 1 or close to 1 suggesting that these conditions could lead to detrimental effects.

No significant difference in estimated Se concentrations for any organ or tissue in thin section of fry was observed by LA-ICP MS between control (Bpc-Fpc), Bpc-Fph and Bph-Fph, with MeHg supplementation (Figs. 1 and 2), which was confirmed by ICP MS quantification. Bidon et al. (2023) also reported that MeHg supplementation had no effect on Se accumulation in juvenile rainbow trout fed for 3 weeks, but only after 6 months of feeding. The same was observed by Amlund et al. (2015) in zebrafish with muscle Se levels ranging from 0.24 to $0.39 \mu\text{g g}^{-1}$ for fish exposed to 5 and $10 \mu\text{g g}^{-1}$ of MeHg, with no significant difference between the feeding conditions.

We can interpret the decrease of Hg content in the kidney of the Bpc-Fpho fry compared to Bpc-Fph and Bph-Fph as a result of SeMet supplementation. However, more replicates should be analyzed to secure a conclusion. A decrease in Hg concentration in the kidney of fish has already been reported with Se supplementation but for different fish species and forms of supplementation: in juvenile white sturgeon supplemented with SeMet and MeHg by Huang et al. (2013) and in minnows supplemented with mercuric chloride and sodium selenite by Cuvinn-Aralar and Furness (1990). In our study, under the conditions of SeMet supplementation, the decrease of Se concentration in the kidney (Fig. 2) might also be attributed to the allocation of Se from the kidney to maintain high Se:Hg molar ratio for protection of the other organs of the trout as also hypothesized for the white sturgeon by Huang et al. (2013).

3.5. Effects of selenium and/or mercury-supplemented diets on copper, zinc and molybdenum localisation in rainbow trout fry

As trace elements can be affected by a change in fish diet, Cu, Zn and Mo, were also quantitatively mapped for possible interaction with Se and Hg supplementation (Antony Jesu Prabhu et al., 2015; Rider et al., 2009). As their localisation and levels were similar for all sample conditions, only quantitative images of one replicate of Bpo-Fpo fry are shown in Fig. 6. Quantitative images of one replicate of the other fry condition for the three elements are presented in Fig. 6SI, and the mean concentrations for each organ are presented in Fig. 7SI.

Molybdenum is an essential trace element for most living organisms and can be found as an enzymatic cofactor involved in metabolism (Kisker et al., 1997). In this study, it was found in the intestine ($0.22 \pm 0.09 \mu\text{g g}^{-1}$), liver ($0.17 \pm 0.04 \mu\text{g g}^{-1}$) and kidney ($0.12 \pm 0.06 \mu\text{g g}^{-1}$) for all feeding conditions. Most replicates were above the LOD, except for kidney in several samples, but were below the LOQ. There were no significant differences between fry conditions for any organ and no trend for variation with diets (data not shown). Therefore, Mo localisation and concentration did not seem to be affected by SeMet and MeHg supplementation.

For all treatments, Cu was detected mainly in the liver of fry at high concentrations (mean of $48 \pm 13 \mu\text{g g}^{-1}$, values from 22 to $85 \mu\text{g g}^{-1}$) and in the intestine ($14 \pm 6 \mu\text{g g}^{-1}$), in agreement with Wischhusen et al. (2021) who reported high signal intensities in this organ in 11-week-fed fry. It was also detected in kidney ($<\text{LOQ}$), the brain and eye periphery but not in the muscle ($<\text{LOD}$). There were no significant differences between the fry conditions for any organ.

In all treatments, Zn was absent from the muscle but was detected in the intestine, kidney, brain, peripheral eye and liver at concentrations close to the LOQ. Its quantification was challenging due to its presence in the substrate material (glass) which resulted in a high LOD ($5 \mu\text{g g}^{-1}$). Zinc levels in Bph-Fph kidney were high, but with an elevated standard deviation, and there were no significant differences between fry conditions for each organ, therefore organic Se and Hg supplementation did not seem to affect Zn localisation.

In summary, for Cu and Zn, there were no diets that led to higher or lower concentrations, meaning that MeHg and SeMet supplementation had no effect on the levels and localisation of these elements, as already reported by Julshamn et al. (1990) and Rider et al. (2009) for organic Se supplementation.

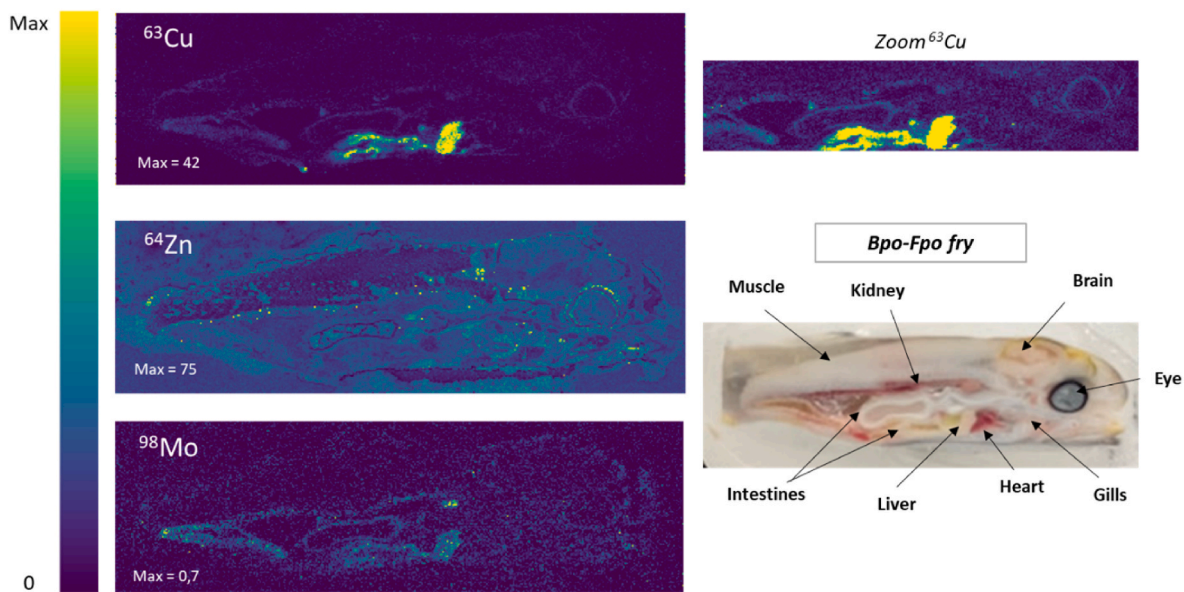


Fig. 6. Example of quantitative images of ^{63}Cu , ^{64}Zn and ^{98}Mo in thin section ($8\ \mu\text{m}$) of a replicate of whole 3-week-fed rainbow trout fry Bpo-Fpo (left) and the photograph of the embedded fry just before sectioning (right). The other feeding conditions of feeding are shown in Fig. 6SI.

4. Conclusion

This work shows that LA-ICP MS is a technique of choice for the quantitative localisation of Se and Hg in the different organs and tissues of rainbow trout fry. Liver, kidney, muscle and intestine displayed the highest concentrations with direct dietary supplementation. It was also found that parental Se supplementation still has an effect on Se levels in 3-week-fed rainbow trout fry, even more with SeMet-supplemented plant diets than with Se from tuna by-products in tuna-based diets. Furthermore, parental Hg was only weakly transferred to the offspring and direct SeMet supplementation in fry diets appeared to reduce kidney Hg content. Se:Hg molar ratios were above 1 in Bpc-Fpho fry suggesting a protective effect against Hg toxicity, but were around or below 1 in non-Se-supplemented Bpc-Fph fry. More replicates should be analyzed for confident conclusions, and other feeding conditions could be studied to provide more information on the metabolism of Se and Hg from parental or direct supplementation with plant-based diets or tuna by-products. Finally, to go further into the understanding of mechanisms of parental offloading and of Se and Hg deposits into fry organs, the determination of the chemical forms of these two elements and their interaction with surrounding biomolecules, would provide key information.

CRedit authorship contribution statement

Laurie Labeyrie: Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Stéphanie Fontagné-Dicharry:** Writing – review & editing, Resources, Methodology. **Sandra Mounicou:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2024.142967>.

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