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In relation to the decrease of selenium (Se) content in aquafeeds, the impact of level and form of parental and dietary Se supplementation was investigated in rainbow trout fry using Laser Ablation-Inductively Coupled Plasma Mass Spectrometry (LA-ICP MS) bioimaging. The offspring of rainbow trout broodstock, fed either a control diet without any Se supplementation (0.3 mg Se/kg diet) or a diet supplemented with Se (0.6 mg Se/kg diet) either as sodium selenite or hydroxy-selenomethionine were sampled at swim-up fry stage or after 11 weeks of cross-feeding. Total body Se levels were influenced by parental Se nutrition in swim-up fry and by direct Se feeding in 11-week fry with higher levels in the Se-supplemented groups compared to the control and the highest levels in the hydroxy-selenomethionine treatment. The Se retention was lower for dietary sodium selenite. SeMet levels increased when Se was provided as hydroxy-selenomethionine. LA-ICP MS maps revealed yolk in swim-up fry and intestine, liver and kidney in 11-week fed fry as tissues with high Se abundance. In swim-up fry, muscle Se was the highest abundant when parents were fed hydroxy-selenomethionine. In 11-week fed fry, muscle Se abundance was higher in the head part of fry fed both Se-supplemented diets, but only in fry fed hydroxy-selenomethionine in the tail part. Liver Se abundance was higher in fry fed sodium selenite compared to the control diet supporting the hypothesis that tissue Se distribution can be influenced by parental and dietary Se forms and levels.

Introduction

In pelleted aquafeeds, fishmeal, as the main natural selenium (Se) source, is increasingly replaced by plant ingredients with low Se content suggesting the need for application of dietary Se supplements.[1,2] Organic selenocysteine (SeCys) and selenomethionine (SeMet) are found in high amounts in fishmeal-based aquafeeds, while plant ingredients rather contain Se in form of SeMet.[3–6] On the market, Se products exist in both organic as well as inorganic forms and these differ fundamentally in their metabolism.[7] In general, the intestinal Se absorption was found to be high, independent from dietary level and form[8,9], but with a higher bioavailability and retention for SeMet compared to the widely used inorganic sodium selenite.[10]

In fish, Se exists mainly as SeMet and SeCys[3], which are also the predominant forms in proteins and selenoproteins, respectively. To be incorporated into selenoproteins, both, organic and inorganic dietary Se needs to be metabolized to hydrogen selenide, the universal Se donor for SeCys tRNA.[11] Selenite can either be reduced to selenide by thioredoxin reductase or by reacting with glutathione.[12] SeMet, on the other hand, can be absorbed by methionine transporters entering the methionine body pool. Then, it is either metabolized via the methionine cycle and transselenation pathway to SeCys and further catabolized to selenide for the mediated incorporation into selenoproteins or directly and randomly incorporated into proteins at the methionine position.[12] The body Se pool is controlled in the liver from where Se can be distributed through the transport and storage protein selenoprotein P, which contains up to 17 SeCys in fish.[13,14]

The tissue distribution under limiting Se conditions follows a hierarchical pattern including a possible redistribution.[15,16] In Atlantic salmon, total body Se concentrations were higher in fish fed a SeMet supplemented diet, but liver Se concentrations were highest when fed sodium selenite under non deficient conditions.[17,18] A similar effect was also observed in rats where Se accumulation in brain and muscle was more pronounced with SeMet compared to sodium selenite.[19]

In fish, Se provided by maternal nutrition during oogenesis represents the available pool during embryonic development. We previously reported that parental Se nutrition affects the amount of Se transferred to the progeny, which was higher in Se-supplemented treatments, especially when supplied as organic Se form.[20] Higher Se levels in the organic Se treatment were accompanied by a modified SeMet to SeCys ratio in swim-up fry before the first feeding. Therefore, it could be assumed that dietary Se form presented by both parental and direct feeding can influence the tissue distribution in the body of fry possibly linked to observed differences in metabolic response.

In this context, the understanding of biological processes involving trace elements can be improved through the study of their distribution in biological tissues. Considering imaging techniques, the spatial resolution (20-50 nm) offered by X-Ray Fluorescence is an advantage to localize metal at subcellular level as demonstrated in fish on otoliths of Sacramento Splittail.[21] However, its sensitivity (100-1000 $ng.g^{-1}$) can be about 2 orders of magnitudes higher than the one of mass spectrometry imaging techniques.[22] Among the latter, Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP MS) is an attractive technique thanks to its sensitivity (10 $ng.g^{-1}$) and its spatial resolution to localize trace elements at the micrometre level within thin sections of biological

tissues or individuals.[23–25] Applications of LA-ICP MS bioimaging are numerous but none concerns Se mapping in whole rainbow trout sections as earlier studies focussed on specific tissues like the otoliths.[26]
The objective of this study was to investigate the effect of different dietary Se forms on the whole-body Se distribution by both parental and direct feeding in rainbow trout fry after cryosectioning and LA-ICPMS imaging.

Material and Methods

Feeding trial with rainbow trout broodstock and fry

All procedures were performed in compliance with the European Directive 2010/63/EU for the protection of animals used for scientific purposes and the French Decree no. 2013-118 for animal experimentation.
The rainbow trout husbandry and diet preparation were previously described.[20,27] Briefly, rainbow trout broodstock were divided into three groups of 25 females and 15 males. The broodstock were fed one of the three experimental diets designed to differ only in Se content (Table 1). The diet Bnc was not supplemented with Se having a basal Se level of 0.3 mg/kg diet (wet weight, dry matter >95%). The broodstock diet Bss was supplemented with 0.3 mg Se/kg diet as sodium selenite to a target total Se concentration of 0.6 mg Se/kg diet, similar to Bso, which was, however, supplemented with 0.3 mg Se/kg diet as hydroxy-selenomethionine (OH-SeMet, Selisseo®). The diets were given for six months prior to spawning. At spawning, oocytes from each spawning female were collected through stripping, pooled and fertilized with a pool of sperm retrieved from males of the same dietary treatment collected at the same day. The eggs were cultivated until swim-up fry stage. At swim-up fry stage, the pooled progeny of each parental treatment was subdivided into three fry feeding groups given one of the three fry diets designed at similar Se levels compared to the broodstock diets (Fnc, Fss, Fso) for 11 weeks (Table 1).

Table 1 Experimental treatments and dietary selenium levels in the rainbow trout feeding trial.

Broodstock diet	Target/analysed Se (mg/kg diet)	Fry diet	Target/analyzed Se (mg/kg diet)	Fry treatment
Bnc; negative control	0.3/0.3	Fnc; negative control	0.3/0.3	BncFnc
		Fss: sodium selenite	0.6/0.5	BncFss
		Fso: OH-SeMet	0.6/0.6	BncFso
Bss: sodium selenite	0.6/0.8	Fnc; negative control	0.3/0.3	BssFnc
		Fss: sodium selenite	0.6/0.5	BssFss
		Fso: OH-SeMet	0.6/0.6	BssFso
Bso: OH-SeMet	0.6/0.7	Fnc; negative control	0.3/0.3	BsoFnc
		Fss: sodium selenite	0.6/0.5	BsoFss
		Fso: OH-SeMet	0.6/0.6	BsoFso

All diets were supplemented with 40 mg/kg $ZnSO_4$ $7H_2O$ and 30 mg/kg $CuSO_4$ $5H_2O$.

Sampling

Sampling took place at swim-up fry stage and at the end of the 11-week feeding period. Rainbow trouts were euthanized with an overdose of benzocaine, weighted and sampled as whole-body fry. Three fries from each treatment were individually spread, wrapped, while the rest of the fish were pooled. All samples were frozen in liquid nitrogen and stored at -80°C.

Total zinc, copper and selenium analysis and selenium speciation

Total zinc (Zn), copper (Cu) and Se as well as SeMet were measured in homogenized swim-up fry and 11-week fed fry. Zn and Cu were determined by inductively coupled plasma mass spectrometry (ICP-MS) as previously described.[28] Total Se was determined using ICP-MS (Agilent series 7500cx) by Ultra Trace Analysis Aquitaine (UT2A, Pau, France) according to Vacchina and Dumont.[29] SeMet was analyzed by liquid chromatography (HPLC, Agilent series 1200) coupled to ICP-MS by UT2A for 11-weeks fed fry[30] and, by the Institut des Sciences Analytiques et de Physico-Chimie pour l'Environnement et les Matériaux (IPREM, Pau, France) for swim-up fry as previously described.[3]

Cryosectioning of fry bodies

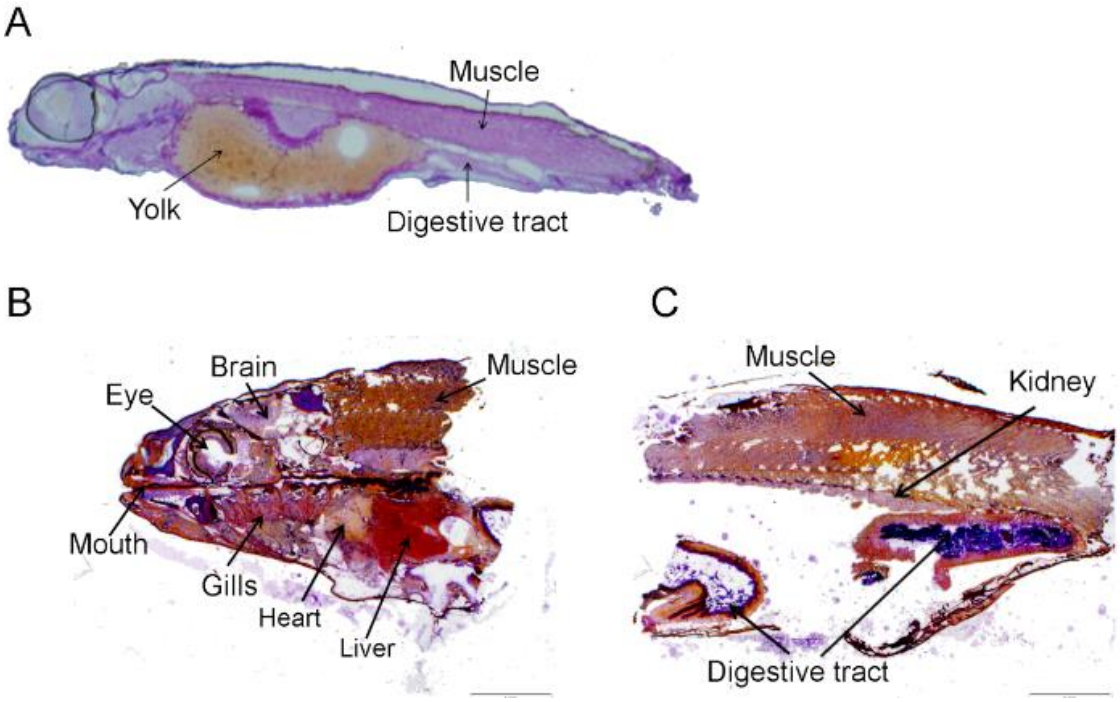

Figure 1 (A) Histologically stained rainbow trout fry at swim-up fry stage or (B) after 11 weeks of external feeding

The 11-week fed fish were cut with a scalpel at the beginning of the dorsal fin, leading to two parts: head and tail, in order to fit the cryosectioning block (2.5 cm diameter). Entire swim-up fry and head and tail parts of the 11-week fed fry were positioned and embedded in a resin (cryomatrix ThermoShadon, 6769006) on the specimen discs and placed in a cryostat (Microm HM 505 E) pre-cooled to -20°C. Then, serial sections of 40 µm were recovered on glass slides, alternating for either histological staining or LA-ICP MS imaging analysis.

Tissue staining

Tissue sections were fixed in 10% formalin solution for 10 min and then rinsed with distilled water for 5 min. They were stained in 1% alcian blue 8GX solution (Sigma-Aldrich, CAS 75881-23-1) for 20 min and rinsed in distilled water for 5 min. The periodic acid Schiff reaction was applied by incubating the slides in 0.5% periodic acid solution for 3 min, rinsing them with running tap water for 5 min and staining with Schiff's reagent (Sigma-Aldrich, 1.09033) for 5 min before rinsing in distilled water. Sections were then stained in Ehrlich' hematoxylin solution (Electron Microscopy Sciences, 26040-05) for 3 min and rinsed in distilled water for 5 min. Staining in Orange G solution was done for 5 min followed by 3 min rinsing in distilled water. Sections were then air-dried for 1h before pictures were taken[31] (Figure 1).

Elemental imaging by Laser Ablation – Inductively coupled Plasma Mass Spectrometry

A 7900x ICP MS (Agilent, Tokyo Japan) detector was used. The instrument was first tuned under liquid sample introduction (with 1 µg L^{-1} Y, Li, Tl, Ce, 2% HNO_3 tuning solution) and H_2 collision cell mode for optimal mass calibration, maximum sensitivity and stability as well as minimal Se background. For the LA-ICP MS analysis, a NWR213 Laser Ablation system (ESI, Freemont, CA, USA) equipped with a TV2 ablation cell was used. A 612 NIST glass reference material was scanned to verify operational conditions and slightly refine if necessary, tuning of argon carrier gas, ion lenses voltages and H_2 collision cell flow rate. The ICP MS was operated in a dry plasma mode, using Ni cones and under H_2 cell gas mode (3.5 $mL.min^{-1}$). Sections of 11-week fed fry were sampled at 100 $\mu m.s^{-1}$ speed with a laser beam of 100 x100 µm, a 20 Hz laser shot frequency and a 4.6 $J.cm^{-1}$ fluency. A distance of 125 µm between each consecutive ablated line was set. In case of swim-up fry, scan speed, laser beam size and distance between 2 consecutives lines were modified to 50 $\mu m.s^{-1}$, 50 x 50 µm, and 75 µm, respectively. The laser-produced aerosol was transported toward the ICP source by He gas at 800 $mL.min^{-1}$. Integration time of 0.4 s was set to monitor both Se isotopes (^{77}Se, ^{78}Se) while copper (^{63}Cu) and zinc (^{64}Zn) isotopes were integrated for 0.7 s, leading to a total sampling time of 0.946 s. Every two days, laser tubing's, ablation cell and ICP MS cones were systematically cleaned to limit instrumental sensitivity decrease. A csv file was recorded for each scanned line and elemental spectrum and images were produced using a homemade program under Python and converted to a pdf file. Element intensity (cps) per pixel was mapped using a color code. The program allowed as well the integration of element intensity on a defined area (muscle for swim-up fry; muscle, liver and kidney for 11-week fed fry) of fish section, enabling to report a number of cps by μm^2.

Statistical analysis

Results are given as means ± standard error of mean (SEM). Data were analyzed using statistical software R (R Core Team). All data were tested for normality and homogeneity. Se and Zn abundances were rank transformed before statistical analysis. In swim-up fry differences between groups were analyzed by one-way ANOVA, while in 11-week fed fry effect of parental and direct Se nutrition were analyzed by two-way ANOVA. The significance level was set to $p < 0.05$ in all analyses and differences between groups were obtained using Tukey's HSD post hoc test. The correlation between and within thin-sections was performed using "Pearson method" (R, psych, version 2.0.9).

Results

Fry performance, body selenium and selenium speciation

The experimental diets were readily accepted by the fry from first feeding onwards. The parental nutritional history had no significant effect on the body weight or feed conversion ratio (FCR) in the 11-week fed fry (Table 2).

Table 2 Performance and Se retention measured in whole-body fry originating from parents fed different selenium diets and then again fed different selenium diets for 11 weeks. Initial body weights at swim-up fry stage: 85 ± 3 mg in Bnc, 82 ± 3 mg in Bss and 85 ± 2 mg in Bso were not significantly different according to one-way ANOVA (broodstock diets: Bnc, control; Bss, + sodium selenite; Bso, +OH-SeMet and fry diets: Fnc, control; Fss, +sodium selenite; Fso, +OH-SeMet).

	Broodstock feeding (BF)			Fry feeding (FF)			p-values		
	Bnc	Bss	Bso	Fnc	Fss	Fso	BF	FF	BFxFF
Body weight [g]	4.7 ± 0.1	4.5 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	0.09	0.40	0.07
FCR[1]	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.44	0.26	0.71
Se retention[2]	54 ± 2	54 ± 2	54 ± 2	59 ± 1[a]	46 ± 1[b]	58 ± 1[a]	0.73	<0.01	0.88

Values are means ± SEM (n=9 rearing tanks of three broodstock or three fry groups). Within rows and for each diet related effect (broodstock feeding, BF and fry feeding, FF), means not sharing a common superscript letter are significantly different according to two-way ANOVA followed by Tukey's HSD.
[1]FCR= dry feed intake [g] / wet weight gain [g]
[2]Se retention= [(Final body weight [g] x final whole body Se [µg/g])-(initial body weight [g] x initial whole body Se [µg/g])] / (total feed intake [g] x dietary Se content [µg/g])

The Se retention in 11-week fed fry was not affected by the parental nutritional history for Se. Similarly, final body weight and FCR were not significantly different according to fry Se nutrition. However, the Se retention was lower, when the fry were fed the Fss diet compared to feeding of Fnc or Fso.Parental feeding with Se-supplemented diets had no significant impact on whole-body Cu or Zn levels of swim-up fry (Table 3) but increased the body Se content with the highest Se levels in the Bso group (Figure 2A). The SeMet concentration was higher in Bso compared to the two other treatments. However, in the Bso as well as in the Bss group, the increase of total Se was also related to the increase of other seleno-compounds including SeCys.
The Zn, Cu and Se analysis in 11-week fed fry revealed no differences in total body Zn, Cu Se or SeMet content according to parental nutritional history (Table 3 and Figure 2B). Significantly higher Se concentrations were found in fry fed Fss compared to Fnc, but the highest Se content was found in fry receiving the Fso diet (Figure 2C). Similar to the Bss treatment in swim-up fry, the Fss treatment showed no increase in SeMet concentration in 11-week fed fry that was only detected in the Fso treatment. In 11-week fed fry no significant interaction between parental and direct feeding on total body Se, SeMet or other organic seleno compound content was detected.

Table 3 Cu and Zn concentrations [mg/kg wet weight] measured in whole-body fry at swim-up fry stage and after 11 weeks of exogenous feeding (broodstock diets: Bnc, control; Bss, +sodium selenite; Bso, +OH-SeMet and fry diets: Fnc, control; Fss: +sodium selenite, Fso: +OH-SeMet).

		Parental effect			Direct feeding effect			P-value		
		Bnc	Bss	Bso	Fnc	Fss	Fso	BF	FF	BFxFF
swim-up fry	**Cu**	0.82 ± 0.08	0.72 ± 0.03	0.81 ± 0.03	na	na	na	0.37	na	na
	Zn	14.2 ± 0.5	14.3 ± 0.6	13.4 ± 1.2	na	na	na	0.69	na	na
11-week fed fry	**Cu**	1.09 ± 0.04	1.30 ± 0.10	1.29 ± 0.06	1.17 ± 0.08	1.19 ± 0.05	1.31 ± 0.09	0.08	0.25	0.36
	Zn	14.3 ± 0.4	13.4 ± 0.4	13.7 ± 0.2	13.9 ± 0.5	13.7 ± 0.2	13.7 ± 0.3	0.20	0.92	0.75

Values are mean ± SEM (n=5 for swim-up fry and n=9 rearing tanks of three broodstock and three fry fry groups). Within rows and for each diet related effect (broodstock feeding, BF and fry feeding, FF), means not sharing a common superscript letter are significantly different according to one- or two-way ANOVA followed by Tukey's HSD. NA= not applicable.

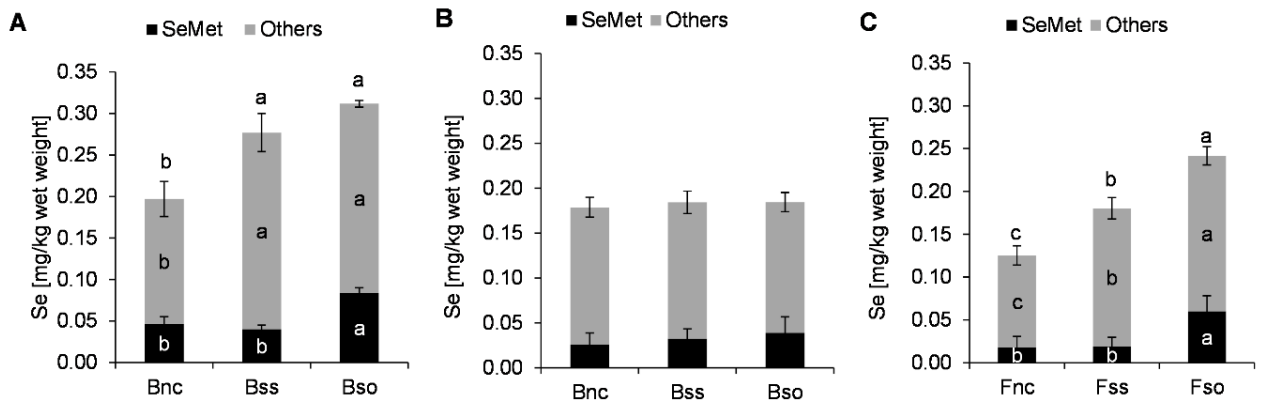

Figure 2 Body concentrations of SeMet and other seleno-compounds (including organic SeCys). (A) Parental selenium in swim-up fry, superscript letters show significant differences according to one-way ANOVA (n=3). (B) Parental effect on Se in 11-week fed fry analyzed by two-way ANOVA (n=9, three broodstock and three fry treatments). (C) Effect of Se feeding in 11-week fed fry analyzed by two-way ANOVA (n=9, three broodstock and three fry treatments). Superscript letters show significant differences in main effect. No significant interaction between selenium of parental and dietary origin was found (broodstock diets: Bnc, control; Bss, + sodium selenite; Bso, +OH-SeMet and fry diets: Fnc, control; Fss, +sodium selenite; Fso, +OH-SeMet).

The Zn, Cu and Se analysis in 11-week fed fry revealed no differences in total body Zn, Cu Se or SeMet content according to parental nutritional history (Table 3 and Figure 2B). Significantly higher Se concentrations were found in fry fed Fss compared to Fnc, but the highest Se content was found in fry receiving the Fso diet (Figure 2C). Similar to the Bss treatment in swim-up fry, the Fss treatment showed no increase in SeMet concentration in 11-week fed fry that was only detected in the Fso treatment. In 11-week fed fry no significant interaction between parental and direct feeding on total body Se, SeMet or other organic seleno compound content was detected.

Localization of trace elements

As shown from example maps in Figure 3, the trace elements Se, Zn and Cu were detected in thin-sections of rainbow trout fry analyzed by LA-ICP MS. The Se abundance visually increased according to the dietary Se treatment, while Zn and Cu abundances

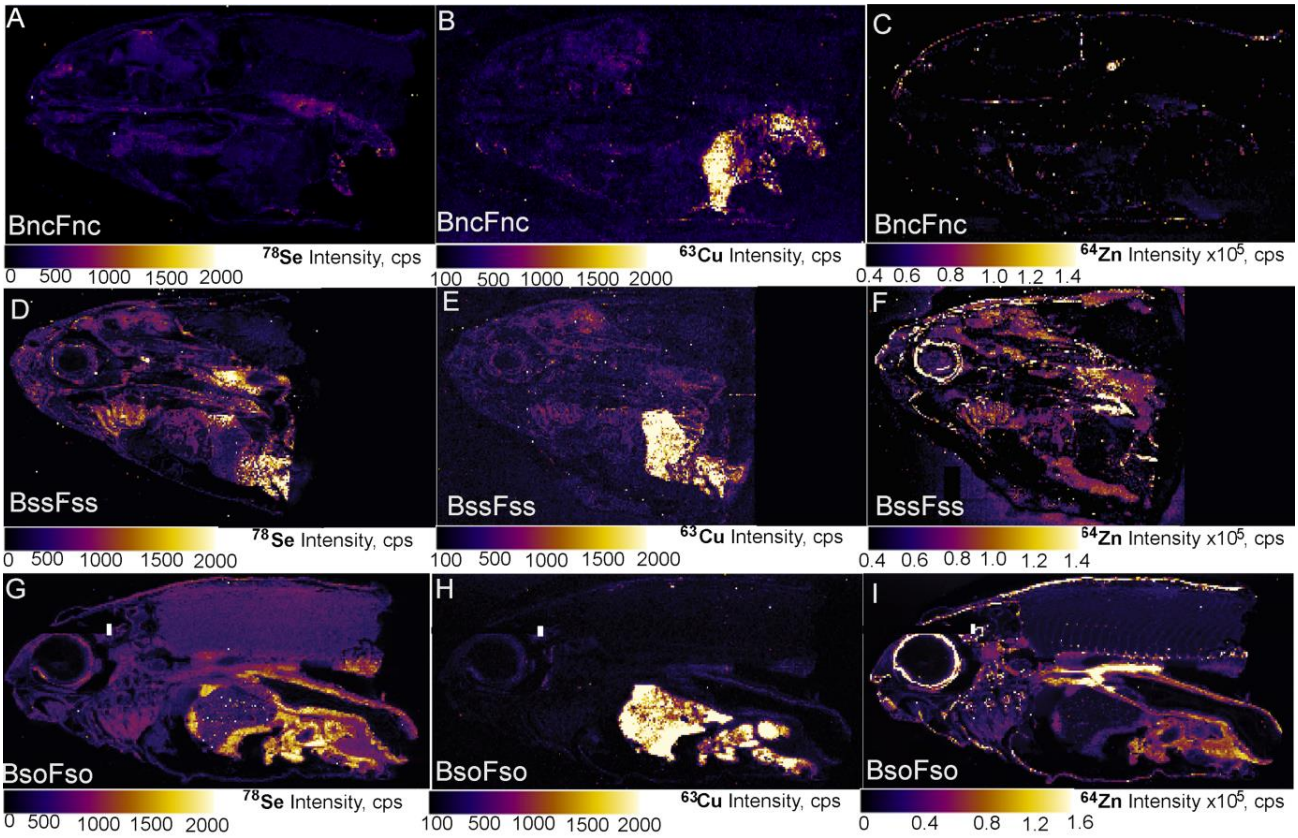

Figure 3 Thin-sections of the head part from three individual rainbow trout fed smilar selenium regimes compared to the parents mapped for Se (A,D,G), Cu (B,E,H) or Zn (C,F,I) (broodstock diets: Bnc, control; Bss, + sodium selenite; Bso, +OH-SeMet and fry diets: Fnc, control; Fss, +sodium selenite; Fso, +OH-SeMet).

were found to be comparable between the dietary treatments. From these images, it was possible to identify liver, kidney and digestive tract as organs with high Se abundance in all dietary treatments. However, the muscle Se abundances, although lower than in the previous organs, were especially high in fry subjected to organic Se treatment. Cu is mainly localized in liver and

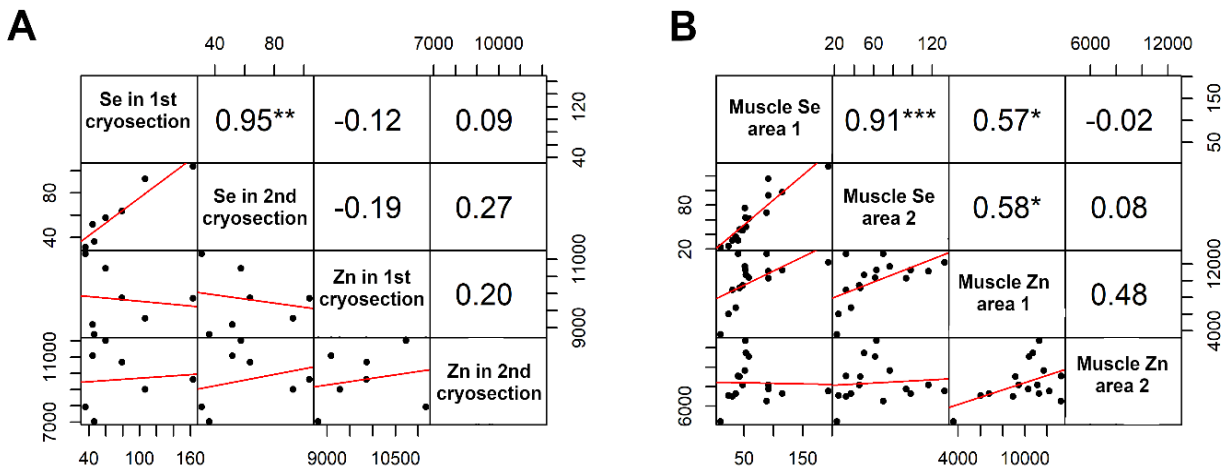

Figure 5 Correlation plots of Se and Zn abundance (cps/µm²) integrated from LA-ICP MS in either two thin-sections of the same individual swim-up fry (A) or two muscle areas within the same thin-section (B).

digestive tract in all dietary treatments. On the other hand, Zn appears more diffuse with higher abundance in the skin and periphery of the eyes in the body with co-localization to Se regardless of the dietary treatment.

Validation of LA-ICP MS methodology for inter section and tissue comparison

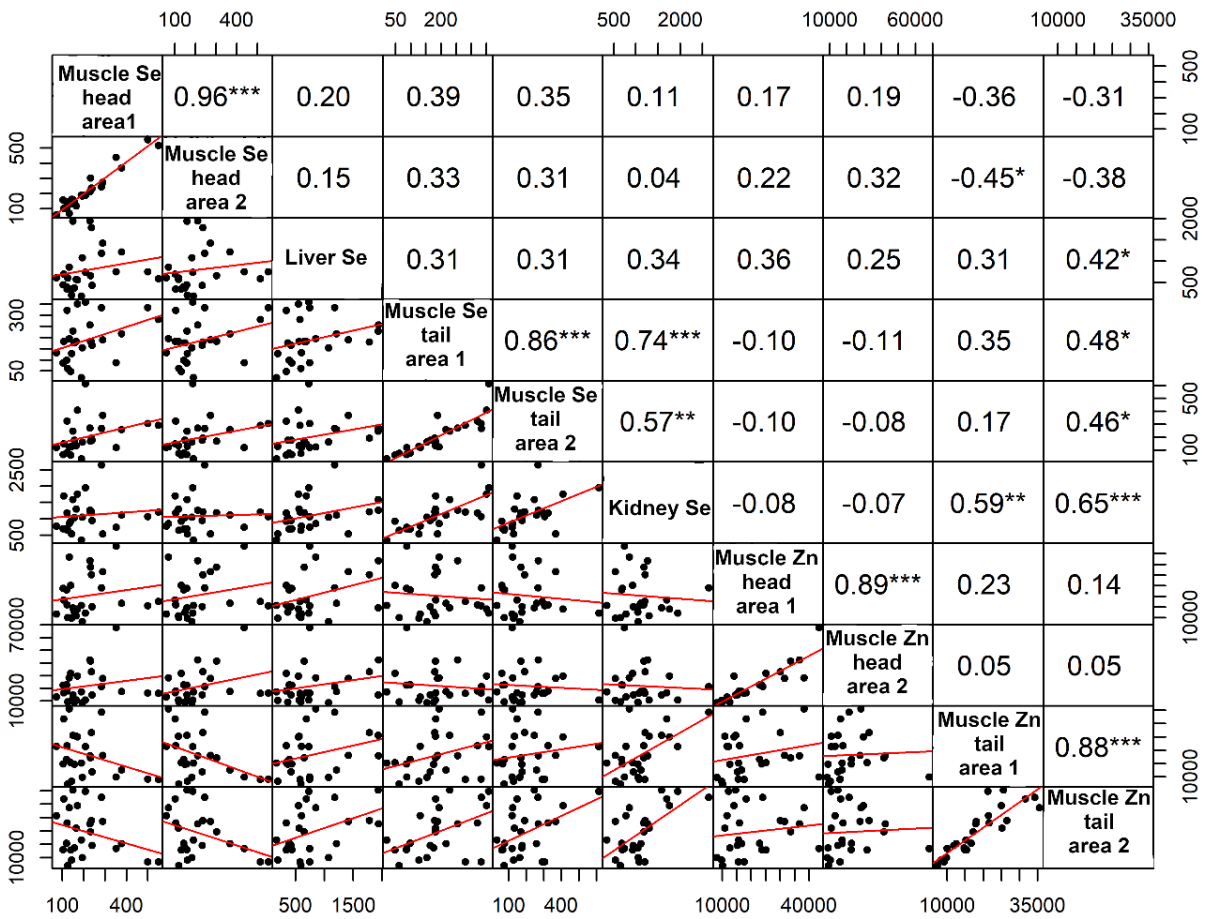

Figure 4 Correlation plot including integrated Se intensity (cps/µm²) from LA-ICP MS in two muscle areas in the head region, two muscle areas in the tail region, the liver and kidney in fry after the 11-week feeding trial. Zn abundance was only determined in corresponding muscle areas.

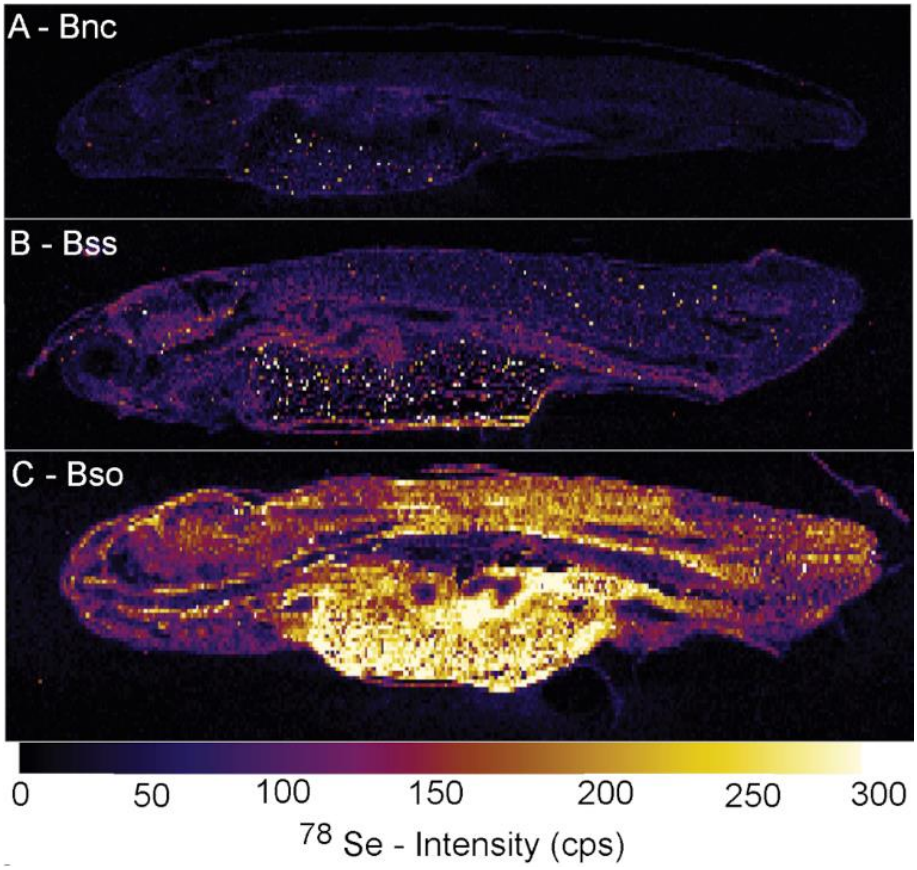

Figure 6 LA-ICP MS maps of Se in rainbow trout swim-up fry originating from parents fed either Bnc (A), Bss (B) or Bso (C) diet (broodstock diets: Bnc, control; Bss, + sodium selenite; Bso, +OH-SeMet).

In order to quantify differences in Se abundance between dietary treatments the Se intensity was integrated over selected tissue areas to achieve an average ratio of intensity to analyzed area (cps/µm^2).

In swim-up fry, the Se abundance within two different thin-sections of the same individual fish (Figure 4A) and within different muscle areas of the same thin-section (Figure 4B) showed a high correlation. However, a drift in sensitivity was observed during analysis accompanied with high variability between two different thin-sections of the same individual.

Therefore, the possibility to use another endogenous element, monitored simultaneously, to standardize Se levels was assessed. In swim-up fry Zn distribution in thin-sections appeared homogenous between treatments by visual examination showing no effect of parental Se treatment. But, as shown in Figure 4 no correlation was detected for Zn between the different thin-sections of the same individual fish, and the correlation between different areas of the same thin-section was weak.

In 11-week fed fry, the muscle Se abundance was strongly correlated in two areas within the same thin-section, but the muscle Se abundance in head and tail parts of the same fish showed only weak correlation (Figure 5). Kidney Se abundance analyzed in tail sections was correlated to muscle Se in the tail, but not in the head section. Zn muscle abundance was strongly correlated within the same thin-section, but not correlated in-between thin-sections (head and tail parts) of the same fish.

Selenium abundance in fry tissues

Visual examination as shown in Figure 6 indicated that the intensity of Se was increased in swim-up fry by parental Se nutrition with the highest Se abundance found in the Bso treatment. Yolk was identified as a Se-rich tissue in all dietary treatments.

The Se appeared evenly distributed within muscle tissue. Muscle Se abundances were found to be higher in Se-supplemented groups, especially in the Bso treatment (Figure 5 and Table 4). The Se/Zn ratio increased with increasing Se abundance.

Table 4 Se and Zn abundance (cps/µm²) in muscle areas of swim-up fry sections integrated from LA-ICP MS

	Bnc	Bss	Bso
Muscle Se	25 ± 5[c]	49 ± 5[b]	102 ± 18[a]
Muscle Zn	6694 ± 1613	9789 ± 1007	9724 ± 327
Muscle Se/Zn	3.7 ± 0.1[c]	5.1 ± 0.1[b]	10.9 ± 1.9[a]

Values are means ± SEM (n=3). Means not sharing a common superscript letter are significantly different according to one-way ANOVA on ranks followed by Tukey's HSD (broodstock diets: Bnc, control; Bss, + sodium selenite; Bso, +OH-SeMet).

After 11 weeks of feeding, in comparison to swim-up fry, the visual examination of LA-ICP MS maps gave no indication for a long-term effect of parental Se nutrition on the body Se abundance or Se distribution in fry (Figure 7).

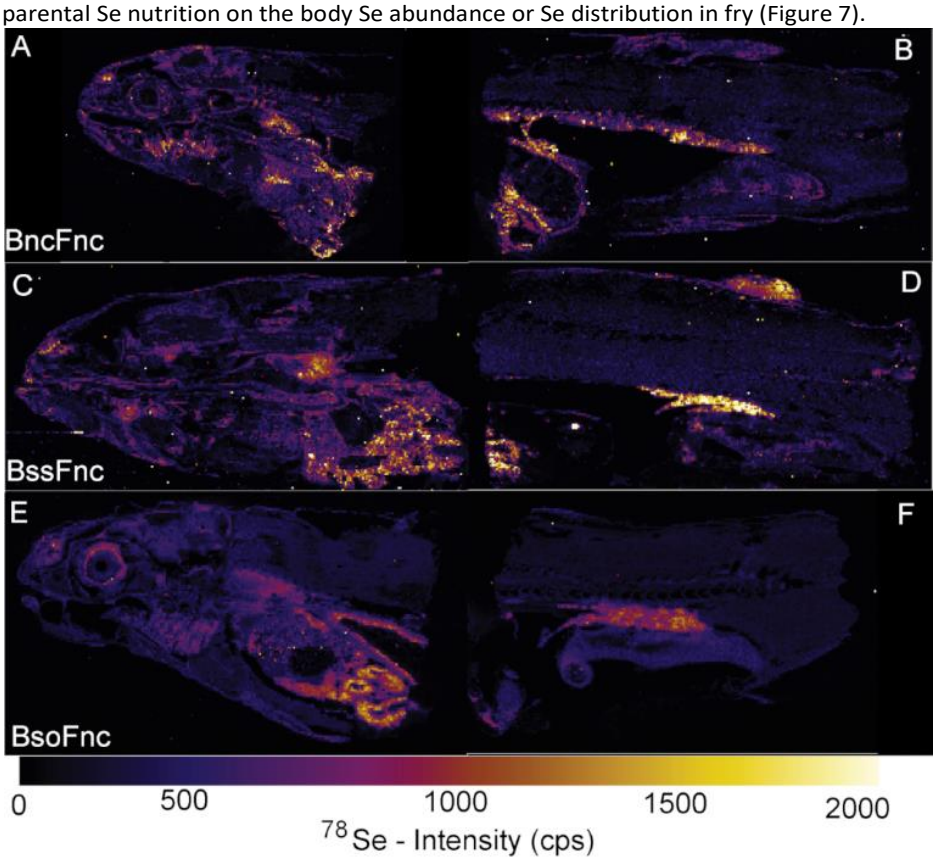

Figure 7 Thin-sections of rainbow trout fry fed the control fry diet Fnc for 11 weeks. LA-ICP MS maps of Se in head and tail of rainbow trout originating from parents fed either Bnc (A+B), Bss (C+D) or Bso (E+F) diet (broodstock diets: Bnc, control; Bss, + sodium selenite; Bso, +OH-SeMet).

In 11-week fed fry the Se abundance in muscle, liver or kidney was not significantly affected by parental Se nutrition (Table 5). The parental effect on the Se/Zn ratio, which was decreased in the Bss compared to Bso treatment, is possibly related to the tendency of higher Zn abundance found in muscle in the head and tail parts of the Bss group compared to both other groups (2-way ANOVA, p=0.08).

The pictures obtained by LA-ICP MS clearly show higher Se abundances in fry fed the Se-supplemented diets Fss and Fso compared to the Fnc treatment (Figure 8). The Se abundance in muscle from the head part and liver was significantly increased in the Fss compared to the Fnc treatment in 11-week fed fry (Table 5). In the Fso treatment, liver Se abundance was not significantly different from Fnc or Fss, but Fso displayed higher Se abundance in muscle from both head and tail parts compared to the Fnc group. The muscle Se/Zn ratio in tail part was also significantly higher in Fss group compared to Fnc. The kidney Se abundance was not significantly different between dietary treatments. No interactive effects between Se from parental and dietary origin on Se or Zn tissue abundance were detected in 11-week fed fry.

Table 5 Se and Zn abundance [(cps/µm²)/1000] in different tissues in head (H) and tail (T) parts of 11-week fed fry integrated from LA-ICP MS (broodstock diets: Bnc, control; Bss, + sodium selenite; Bso, +OH-SeMet and fry diets: Fnc, control; Fss, +sodium selenite; Fso, +OH-SeMet).

	Broodstock feeding			Feeding feeding			P-value		
	Bnc	Bss	Bso	Fnc	Fss	Fso	BF	FF	BFxFF
Selenium									
Muscle (H)	0.20 ± 0.03	0.19 ± 0.03	0.27 ± 0.06	0.14 ± 0.02^{b}	0.23 ± 0.03^{a}	0.27 ± 0.06^{a}	0.43	**0.03**	0.13
Muscle (T)	0.21 ± 0.05	0.16 ± 0.04	0.22 ± 0.03	0.12 ± 0.02^{b}	0.19 ± 0.03^{ab}	0.28 ± 0.04^{a}	0.24	**0.01**	0.59
Liver	0.95 ± 0.18	0.72 ± 0.16	0.69 ± 0.18	0.45 ± 0.07^{b}	1.22 ± 0.21^{a}	0.75 ± 0.11^{ab}	0.21	**0.01**	0.37
Kidney	1.12 ± 0.25	1.13 ± 0.14	1.03 ± 0.08	0.87 ± 0.13	1.30 ± 0.22	1.19 ± 0.15	0.88	0.14	0.87
Zinc									
Muscle (H)	20 ± 2	27 ± 5	16 ± 2	19 ± 3	29 ± 5	17 ± 2	0.08	0.13	0.12
Muscle (T)	23 ± 3	19 ± 3	14 ± 2	19 ± 3	20 ± 3	18 ± 3	0.08	0.86	0.86
Se/Zn*1000									
Muscle (H)	11 ± 2^{ab}	8 ± 1^{b}	18 ± 4^{a}	9 ± 2^{b}	10 ± 2^{ab}	17 ± 3^{a}	**0.01**	**0.03**	0.38
Muscle (T)	9 ± 2^{b}	8 ± 1^{b}	18 ± 4^{a}	7 ± 2^{c}	10 ± 1^{b}	18 ± 3^{a}	**<0.01**	**0.01**	0.03

Values are means ± SEM (n=9 rearing tanks of three broodstock or three fry groups). Within rows and for each diet related effect (broodstock feeding, BF and fry feeding, FF), means not sharing a common superscript letter are significantly different according to two-way ANOVA on ranks followed by Tukey's HSD.

Discussion

The quantification of Se abundance: Methodological considerations

The results obtained by LA-ICP MS imaging support the hypothesis that tissue Se distribution in rainbow trout is not random with localization mainly in liver, kidney, muscle and digestive tract depending on the parental and dietary Se treatment. However, to determine differences between tissues or dietary treatments it seems inevitable to develop tools that allow the quantification of the abundance of the detected trace elements beside the visual examination. Therefore, we integrated the Se intensity of defined areas to obtain a representative Se abundance in selected tissues. For swim-up fry, the high correlation between different areas of the same tissue indicates that the analysis and data interpretation is valid for Se, while Zn abundance only weakly correlates, which might be related to a non-homogenous Zn distribution in muscle. A high correlation of Se abundance between two sections of the same swim-up fry is promising for their comparison. However, in 11-week fed fry, the correlation of Se abundances between two thin-sections of the same fish was only weak, which might be either due to differences within the tissue by changing Se levels in head and tail area of the fish or due to instrumental sensitivity drift causing high variability between consecutively analyzed thin-sections. The possible sensitivity drift during one section imaging could be more noticeable for 11-week fed fry which are bigger in size than the swim-up fry, requiring thus longer analysis time and leading to a more pronounced clogging of the system at the end of imaging. Such sensitivity drift might be compensated by using an internal standard homogeneously distributed in the sample for normalization purpose. The main difficulty in this study was the size of the 11-week fed fry samples (approx. 2 x 3 cm after cutting into 2 parts), which requires to cover a large surface with an internal standard pending further developments. To reduce the impact of variability between sections our idea was to utilize Zn as an internal standard that should be more similar between dietary groups than Se. Even if in swim-up fry, the Se/Zn ratios tended to increase with Se absolute abundance, this approach turned less promising due to the variability of Zn distribution particularly in 11-week fed fry body. In addition, strength of the methodology is its capability to map simultaneously other endogenous elements like the essential micronutrients Cu and Zn. In this study, the dietary Se treatment seems not to promote Cu and Zn redistribution in fry body letting us suppose that their biological functions are preserved. In contrast, another study highlights the correlation of Se and Zn accumulation in eyes and pigment containing tissue of zebrafish larvae exposed to toxic Se levels.[32] At the dietary Se levels utilized in the present study, Zn is also observed in pigments and eyes of 11-week fed fry, though with higher abundance compared to Se. The clear link between Zn and Se deposition in rainbow trout fry deserves further investigation in future studies, especially when considering that effects of dietary Se can be also driven by changes in other trace elements.

Short and long-term effect of parental Se nutrition on Se deposition in fry

With the decreasing Se levels in commercial fish diets, recent studies highlighted the beneficial effect of dietary Se supplementation on the antioxidant system in fish.[33] This should be especially important at sensitive stages like reproduction.[20] Our results of bioimaging and Se analysis show that feeding Se-supplemented diets to rainbow trout broodstock increased the body Se levels in the progeny in a dose and form dependent manner. The highest Se levels were found in swim-up fry fed diets supplemented with OH-SeMet, which possibly relates to the superior bioavailability of organic compared to inorganic Se compounds.[10] LA-ICP MS imaging of swim-up fry of the OH-SeMet group indicated also higher muscle Se abundance, meaning thus

that the developing fry also transferred and deposited more available Se from yolk to muscle. Likewise, as shown in zebrafish broodstock fed high SeMet levels (30 mg Se/kg diet) by X-ray fluorescence imaging, Se can accumulate in the yolk but also in the eye and other tissues of zebrafish larvae.[34] This is similar to what is known in poultry where in fertilized eggs both embryonic and extra-embryonic Se levels were increased, especially when hens were fed organic Se supplied as Se-enriched yeast.[35] In chicken, higher Se levels in broodstock diets increased Se concentrations in yolk, albumen, liver, and breast muscle of the developing embryo, but irrespective of the Se level presented to the breeders, embryonic liver and breast muscle concentrations were higher when fed SeMet compared to sodium selenite.[36] However, the importance of Se in the metabolism mainly relates to the activity of selenoproteins, where Se is incorporated as SeCys. Therefore, it would have been interesting to determine, if yolk and muscle Se in the progeny was protein bound considering that whole body SeMet levels were increased in the OH-SeMet treatment. A protein profiling in eggs and muscle tissue coupled to an imaging technique might be a promising approach in future studies to answer such questions. Nevertheless, in embryonic chicken the parental Se transfer was associated with higher glutathione peroxidase activity and an enhanced antioxidant system[37] and also we have previously demonstrated that increased Se levels in the progeny of rainbow trout by parental OH-SeMet feeding resulted in increased glutathione peroxidase mRNA levels and activity[20]. Together, these results suggest that parental feeding with organic Se might be more beneficial for the developing progeny compared to inorganic Se forms. However, in the long term, the effect of parental Se nutrition on body Se levels was no more detectable probably due to the strong effect of fry Se nutrition. Indeed, the quantity of parental Se represents potentially only 1 to 5% of total Se in 11-week fed fry.

Effect of direct Se feeding on the Se deposition in whole-body fry

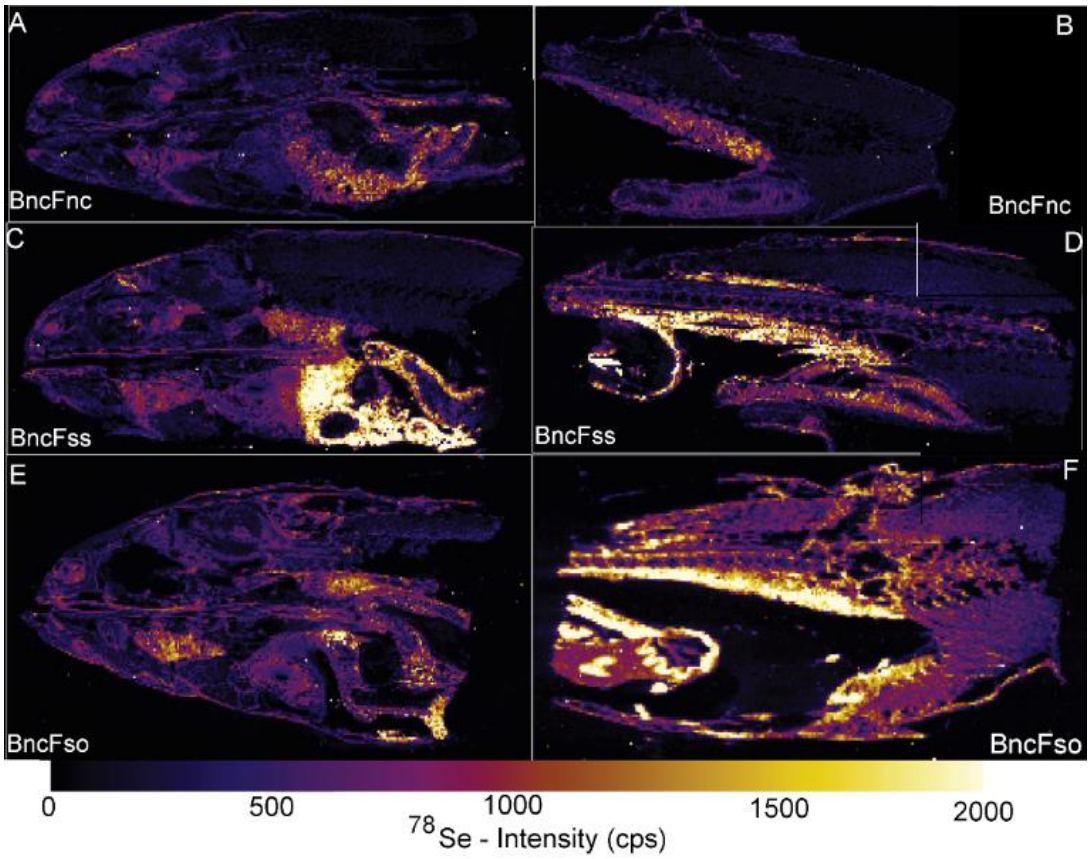

Figure 8 Thin-sections of rainbow trout fry originating from parents fed the broodstock control diet Bnc for 6 months prior to spawning. LA-ICP MS maps of Se in head and tail parts of rainbow trout fed either the Fnc diet (A+B), the Fss diet (C+D) or the Fso diet (E+F). (fry diets: Fnc, control; Fss, +sodium selenite; Bso, +OH-SeMet).

Similar to previous studies, we detected that dietary Se supplementation increased the body Se levels in rainbow trout fry.[3] The Se retention (58%) in fry fed the basal diet or the diet supplemented with OH-SeMet was not found to differ. This might be related to the composition of basal diet as it has been previously shown that SeMet account for high amounts of the total Se in these plant-based diets.[3,38] Similarly, retention of 58-61% of dietary Se supplied as L-SeMet has been reported in Atlantic salmon post-smolts.[18] On the other hand, the Se retention was lowered to 46% when fry were fed diets supplemented with sodium selenite in accordance with other terrestrial animal and fish studies where inorganic Se sources have been described to be lower retained[39,40] with Se retention as low as 30-38% in Atlantic salmon[18]. In a previous study with rainbow trout fry fed plant-based diets

supplemented with Se-enriched yeast, SeMet was found to represent 50% of total Se in whole-body fry[3] whereas in the present study, SeMet represented only 25% of total Se in whole-body fry fed OH-SeMet. This lower SeMet proportion is probably indicative of reduced Se storage. However, in the present study, whole-body total Se of 11-week fed fry ranged from 120 to 260 µg/kg wet weight similarly to previous study in rainbow trout fry fed plant-based diets supplemented with 0.3 mg Se/kg diet supplied either as sodium selenite or Se-enriched yeast.[33] Fish body Se homeostasis has been used in Atlantic salmon post-smolt to define dietary Se requirement[18] and body Se levels lower than 200 µg/kg were indicative of dietary Se deficiency. So, in the present study the control diet Fnc might be considered as Se-deficient but also the sodium-selenite supplemented diet Fss according to whole-body levels. However, glutathione peroxidase activity that has been used as a specific response criterion to estimate dietary Se requirements of fish was higher in fry fed sodium selenite compared to the control treatment, the OH-SeMet treatment being intermediate[27]. In a previous study in rainbow trout fry with dietary Se levels ranging from 0.5-0.9 mg Se/kg diet, the non-supplemented diets containing 0.5 mg Se/kg diet were found to be Se deficient according to glutathione peroxidase activity.[33] So the question remains if the Se supplemented diets used in the present study containing 0.5-0.6 mg Se/kg diet, might be considered as sub-optimal for rainbow trout fry.

Effect of Se nutrition on the Se deposition in tissues of rainbow trout fry

Similar to earlier studies in rainbow trout juveniles of larger size[9], we detected high Se levels in liver and kidney of 11-week fed fry by means of bioimaging. In the present study, quite high levels were also noticed in the digestive tract. In juvenile rainbow trout fed high levels of SeMet, a marked increase of Se deposition was also observed in all major tissues including the brain[41], which was not noticed in the present study probably due to the use of lower dietary levels. In the same study[41], SeMet was found to be the predominant form of Se (up to 40%) in all of the tissues including liver and kidney involved in Se handling. The distribution of Se for the production of selenoproteins to different organs is mediated by the liver.[12] In this organ, integration of LA-ICP MS measurements gave the highest Se abundance in the sodium selenite treatment as described previously in rainbow trout juveniles[42]. It might suggest superior availability of selenite for inclusion in selenoproteins as described in pigs by serum glutathione peroxidase levels.[43] Hilton et al.[44] found that in rainbow trout liver Se levels rose disproportionally to dietary Se levels. While liver and kidney Se levels readily increased, when rainbow trout were fed diets supplemented with sodium selenite up to a dietary concentration of 0.35 mg Se/kg dry feed, at higher dietary Se levels the liver Se concentrations disproportionally increased compared to kidney levels. Similar results for differentially deposition of Se in liver and kidney were observed in rainbow trout juveniles fed SeMet at levels of 10 and 40 mg/kg diet.[45] In the present study, the increase in kidney Se abundance as observed by LA-ICP MS imaging in Se-supplemented treatments was not significant. In accordance with these findings it could be suggested that at physiological basal Se levels (higher than 0.35 mg Se/kg diet), dietary Se supplementation with sodium selenite is beneficial for selenoprotein production in liver tissue, but at higher levels (above 1.25 mg Se/kg diet), liver Se accumulation might induce cytotoxicity that cannot be regulated through urinary excretion.

In the present study, bioimaging revealed that both OH-SeMet and sodium selenite nutrition increased muscle Se abundance in rainbow trout fry, but OH-SeMet was more effective. In Atlantic salmon juveniles, muscle Se levels were found to increase with feeding diets supplemented with SeMet and sodium selenite with a SeMet abundance in muscle tissue of over 90% in both treatments.[38] It cannot be assumed that sodium selenite is transformed to SeMet[12], but rather that the SeMet of the basal level is transferred to the muscle tissue in the sodium selenite treatment to larger amounts compared to the negative control, which is in accordance with the present results that only in the organic Se treatment body SeMet levels increased. However, on whole body level we also detected a significant increase of other seleno-compounds including SeCys in the organic Se treatment that could suggest a selenoprotein production or deposition in muscle tissue. Indeed, Wand et al.[46] found that a dietary supplementation with Se-yeast increased mRNA levels of several selenoproteins in muscle in comparison to liver where mainly SelP was affected. A combined study on tissue Se and selenoprotein levels might help to further elucidate the biological relevance of tissue Se abundance and how different Se forms are further metabolized.

Conclusions

LA-ICP MS bioimaging demonstrated that tissue Se distribution could change pending on parental and dietary Se treatment in rainbow trout fry. While the Se levels before first feeding were influenced by parental Se nutrition, in the long-term it was dominated by the feeding regime. This study supports that muscle and liver Se concentration might be good indicators of the Se loading, depending on dietary Se form. Imaging analysis of the entire fry revealed that OH-SeMet efficiently raised the muscle Se content in swim-up fry through parental nutrition and by direct feeding later in life.

Author Contributions

Conceptualization, S.F.-D., S.J.K., B.B., P.-A.G.; methodology, C.A., M.B., B.B., S.F.-D., S.M.; software, G.V.; formal analysis, P.W.; investigation, P.W.; C.A.; M.B., S.F.-D., S.M.; writing original draft, P.W.; writing review and editing, P.A.J.P.; P.-A.G., B.F., S.M., S.F.-D.; visualization, P.W., S.M.; supervision, B.F., S.F.-D.; S.M.; project administration, S.F.-D.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1 C. M. Weekley and H. H. Harris, Which form is that? The importance of selenium speciation and metabolism in the prevention and treatment of disease, *Chemical Society Reviews*, 2013, **42**, 8870–8894.
2 A. G. J. Tacon and M. Metian, Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects, *Aquaculture*, 2008, **285**, 146–158.
3 S. Godin, S. Fontagné-Dicharry, M. Bueno, P. Tacon, P. A. Jesu Prabhu, S. Kaushik, F. Médale and B. Bouyssiere, Influence of dietary selenium species on selenoamino acid levels in rainbow trout, *Journal of agricultural and food chemistry*, 2015, **63**, 6484–6492.
4 V. Sele, R. Ørnsrud, J. J. Sloth, M. H. Berntssen and H. Amlund, Selenium and selenium species in feeds and muscle tissue of Atlantic salmon, *Journal of Trace Elements in Medicine and Biology*, 2018, **47**, 124–133.
5 J. G. Bell and C. B. Cowey, Digestibility and bioavailability of dietary selenium from fishmeal, selenite, selenomethionine and selenocystine in Atlantic salmon (Salmo salar), *Aquaculture*, 1989, **81**, 61–68.
6 M. H. Berntssen, M. Betancor, M. J. Caballero, M. Hillestad, J. Rasinger, K. Hamre, V. Sele, H. Amlund and R. Ørnsrud, Safe limits of selenomethionine and selenite supplementation to plant-based Atlantic salmon feeds, *Aquaculture*, 2018, **495**, 617–630.
7 G. F. Combs and S. B. Combs, The nutritional biochemistry of selenium, *Annual review of nutrition*, 1984, **4**, 257–280.
8 S. C. Vendeland, J. A. Butler and P. D. Whanger, Intestinal absorption of selenite, selenate, and selenomethionine in the rat, *The Journal of Nutritional Biochemistry*, 1992, **3**, 359–365.
9 J. W. Hilton, P. V. Hodson and S. J. Slinger, Absorption, distribution, half-life and possible routes of elimination of dietary selenium in juvenile rainbow trout (Salmo gairdneri), *Comparative Biochemistry and Physiology Part C: Comparative Pharmacology*, 1982, **71**, 49–55.
10 C. Wang and R. T. Lovell, Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than an inorganic selenium source, sodium selenite, in diets for channel catfish (Ictalurus punctatus), *Aquaculture*, 1997, **152**, 223–234.
11 L. V. Papp, J. Lu, A. Holmgren and K. K. Khanna, From selenium to selenoproteins: synthesis, identity, and their role in human health, *Antioxidants & redox signaling*, 2007, **9**, 775–806.
12 R. F. Burk and K. E. Hill, Regulation of Selenium Metabolism and Transport, *Annual Review of Nutrition*, 2015, **35**, 109–134.
13 M. A. N. Pohl, T. Wang, T. Pohl, J. Sweetman, S. A. M. Martin and C. J. Secombes, Four selenoprotein P genes exist in salmonids: Analysis of their origin and expression following Se supplementation and bacterial infection, *PLoS One*, , DOI:10.1371/journal.pone.0209381.
14 R. F. Burk, K. E. Hill, A. K. Motley, L. M. Austin and B. K. Norsworthy, Deletion of selenoprotein P upregulates urinary selenium excretion and depresses whole-body selenium content, *Biochimica et Biophysica Acta (BBA) - General Subjects*, 2006, **1760**, 1789–1793.
15 U. Schweizer, L. Schomburg and J. Köhrle, in *Selenium: Its Molecular Biology and Role in Human Health*, eds. D. L. Hatfield, U. Schweizer, P. A. Tsuji and V. N. Gladyshev, Springer International Publishing, Cham, 2016, pp. 261–274.
16 D. Behne and T. Höfer-Bosse, Effects of a Low Selenium Status on the Distribution and Retention of Selenium in the Rat, *J Nutr*, 1984, **114**, 1289–1296.
17 M. Lorentzen, A. Maage and K. Julshamn, Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon (Salmo salar), *Aquaculture*, 1994, **121**, 359–367.
18 Prabhu. P. Antony Jesu, E. Holen, M. Espe, M. S. Silva, M.-H. Holme, K. Hamre, E.-J. Lock and R. Waagbø, Dietary selenium required to achieve body homeostasis and attenuate pro-inflammatory responses in Atlantic salmon post-smolt exceeds the present EU legal limit, *Aquaculture*, 2020, 735413.
19 P. D. Whanger and J. A. Butler, Effects of Various Dietary Levels of Selenium as Selenite or Selenomethionine on Tissue Selenium Levels and Glutathione Peroxidase Activity in Rats, *J Nutr*, 1988, **118**, 846–852.

20 P. Wischhusen, M. Parailloux, P.-A. Geraert, M. Briens, M. Bueno, S. Mounicou, B. Bouyssiere, P. Antony Jesu Prabhu, S. J. Kaushik, B. Fauconneau and S. Fontagné-Dicharry, Effect of dietary selenium in rainbow trout (Oncorhynchus mykiss) broodstock on antioxidant status, its parental transfer and oxidative status in the progeny, *Aquaculture*, 2019, **507**, 126–138.
21 R. C. Johnson, A. R. Stewart, K. E. Limburg, R. Huang, D. Cocherell and F. Feyrer, Lifetime Chronicles of Selenium Exposure Linked to Deformities in an Imperiled Migratory Fish, *Environ. Sci. Technol.*, 2020, **54**, 2892–2901.
22 B. De Samber, G. Silversmit, R. Evens, K. De Schamphelaere, C. Janssen, B. Masschaele, L. Van Hoorebeke, L. Balcaen, F. Vanhaecke, G. Falkenberg and L. Vincze, Three-dimensional elemental imaging by means of synchrotron radiation micro-XRF: developments and applications in environmental chemistry, *Anal Bioanal Chem*, 2008, **390**, 267–271.
23 D. Pozebon, G. L. Scheffler and V. L. Dressler, Recent applications of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for biological sample analysis: a follow-up review, *J. Anal. At. Spectrom.*, 2017, **32**, 890–919.
24 J. S. Becker, J. S. Becker, M. V. Zoriy, J. Dobrowolska and A. Matusch, Imaging mass spectrometry in biological tissues by laser ablation inductively coupled plasma mass spectrometry, *European Journal of Mass Spectrometry*, 2007, **13**, 1–6.
25 T. J. Stewart, Across the spectrum: integrating multidimensional metal analytics for in situ metallomic imaging, *Metallomics*, 2019, **11**, 29–49.
26 V. P. Palace, N. M. Halden, P. Yang, R. E. Evans and G. Sterling, Determining Residence Patterns of Rainbow Trout Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS) Analysis of Selenium in Otoliths, *Environ. Sci. Technol.*, 2007, **41**, 3679–3683.
27 P. Wischhusen, L. Larroquet, T. Durand, C. Oger, J.-M. Galano, A. Rocher, C. Vigor, P. Antony Jesu Prabhu, V. Véron, M. Briens, J. Roy, S. J. Kaushik, B. Fauconneau and S. Fontagné-Dicharry, Oxidative stress and antioxidant response in rainbow trout fry exposed to acute hypoxia is affected by selenium nutrition of parents and during first exogenous feeding, *Free Radical Biology and Medicine*, , DOI:10.1016/j.freeradbiomed.2020.05.006.
28 P. A. J. Prabhu, E.-J. Lock, G.-I. Hemre, K. Hamre, M. Espe, P. A. Olsvik, J. Silva, A.-C. Hansen, J. Johansen, N. H. Sissener and R. Waagbø, Recommendations for dietary level of micro-minerals and vitamin D3 to Atlantic salmon (Salmo salar) parr and post-smolt when fed low fish meal diets, *PeerJ*, 2019, **7**, e6996.
29 V. Vacchina and J. Dumont, in *Selenoproteins*, Springer, 2018, pp. 145–152.
30 V. Vacchina, K. Bierla, J. Szpunar and R. Lobinski, in *Selenoproteins*, Springer, 2018, pp. 153–162.
31 ECP, INRAE, https://doi.org/10.15454/1.5572402068944548E12, 2018.
32 N. V. Dolgova, M. J. Hackett, T. C. MacDonald, S. Nehzati, A. K. James, P. H. Krone, G. N. George and I. J. Pickering, Distribution of selenium in zebrafish larvae after exposure to organic and inorganic selenium forms, *Metallomics*, 2016, **8**, 305–312.
33 S. Fontagné-Dicharry, S. Godin, H. Liu, P. A. J. Prabhu, B. Bouyssiere, M. Bueno, P. Tacon, F. Médale and S. J. Kaushik, Influence of the forms and levels of dietary selenium on antioxidant status and oxidative stress-related parameters in rainbow trout (Oncorhynchus mykiss) fry, *British Journal of Nutrition*, 2015, **113**, 1876–1887.
34 S. Choudhury, J. K. Thomas, N. J. Sylvain, O. Ponomarenko, R. A. Gordon, S. M. Heald, D. M. Janz, P. H. Krone, I. Coulthard, G. N. George and I. J. Pickering, Selenium preferentially accumulates in the eye lens following embryonic exposure: a confocal X-ray fluorescence imaging study, *Environ. Sci. Technol.*, 2015, **49**, 2255–2261.
35 N. Paton, A. Cantor, A. Pescatore, M. Ford and C. Smith, The effect of dietary selenium source and level on the uptake of selenium by developing chick embryos, *Poultry Science*, 2002, **81**, 1548–1554.
36 D. Yuan, X. Zhan and Y. Wang, Effects of selenium sources and levels on reproductive performance and selenium retention in broiler breeder, egg, developing embryo, and 1-day-old chick, *Biological trace element research*, 2011, **144**, 705–714.
37 X. Xiao, D. Yuan, Y.-X. Wang and X.-A. Zhan, The protective effects of different sources of maternal selenium on oxidative stressed chick embryo liver, *Biological trace element research*, 2016, **172**, 201–208.
38 V. Sele, R. Ørnsrud, J. J. Sloth, M. H. Berntssen and H. Amlund, Selenium and selenium species in feeds and muscle tissue of Atlantic salmon, *Journal of Trace Elements in Medicine and Biology*, 2018, **47**, 124–133.
39 I. Yoon, T. M. Werner and J. M. Butler, Effect of Source and Concentration of Selenium on Growth Performance and Selenium Retention in Broiler Chickens, *Poultry Science*, 2007, **86**, 727–730.
40 P. Antony Jesu Prabhu, J. W. Schrama and S. J. Kaushik, Mineral requirements of fish: a systematic review, *Reviews in Aquaculture*, 2016, **8**, 172–219.
41 S. Misra, D. Peak, N. Chen, C. Hamilton and S. Niyogi, Tissue-specific accumulation and speciation of selenium in rainbow trout (Oncorhynchus mykiss) exposed to elevated dietary selenomethionine, *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 2012, **155**, 560–565.
42 S. A. Rider, S. J. Davies, A. N. Jha, A. A. Fisher, J. Knight and J. W. Sweetman, Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (Oncorhynchus mykiss): implications on selenium status and health responses, *Aquaculture*, 2009, **295**, 282–291.
43 D. C. Mahan and N. A. Parrett, Evaluating the efficacy of selenium-enriched yeast and sodium selenite on tissue selenium retention and serum glutathione peroxidase activity in grower and finisher swine, *J Anim Sci*, 1996, **74**, 2967–2974.
44 J. W. Hilton, P. V. Hodson and S. J. Slinger, The requirement and toxicity of selenium in rainbow trout (Salmo gairdneri), *The Journal of nutrition*, 1980, **110**, 2527–2535.
45 A. Jamwal, Y. Saibu, T. C. MacDonald, G. N. George and S. Niyogi, The effects of dietary selenomethionine on tissue-specific accumulation and toxicity of dietary arsenite in rainbow trout (Oncorhynchus mykiss) during chronic exposure, *Metallomics*, 2019, **11**, 643–655.
46 L. Wang, X. Zhang, L. Wu, Q. Liu, D. Zhang and J. Yin, Expression of selenoprotein genes in muscle is crucial for the growth of rainbow trout (Oncorhynchus mykiss) fed diets supplemented with selenium yeast, *Aquaculture*, 2018, **492**, 82–90.