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1 Effect of dietary Se in rainbow trout (*Oncorhynchus mykiss*) broodstock on antioxidant  
2 status, its parental transfer and oxidative status in the progeny

3

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20

21 **Abstract**

22 We studied the effects of supplementing a plant-ingredient based rainbow trout broodstock  
23 diet with different forms of selenium (Se) on reproductive performance, parental Se transfer  
24 and antioxidant metabolism in their progeny before first-feeding. Three groups of rainbow  
25 trout were fed a diet either without selenium supplementation (NC, basal Se level: 0.3 ppm)  
26 or supplemented with 0.3 ppm Se either in the form of sodium selenite (SS) or as hydroxy-  
27 selenomethionine (SO, dietary Se level: 0.7 ppm) over a 6-month period prior to spawning.  
28 In Se-supplemented groups, the total number of females spawning was significantly higher  
29 compared to the negative control group and the females fed SO began to spawn earlier  
30 compared to females fed SS or NC. Total Se concentrations were significantly higher in  
31 female muscle of SO group. Higher Se concentrations in the oocytes of both Se-  
32 supplemented groups confirmed a maternal transfer of Se, while total Se concentrations in  
33 milt samples were not significantly different between dietary groups. There was no effect on  
34 glutathione peroxidase (GPX) or other antioxidant enzyme activity in the liver of female or  
35 male broodstock, whereas in the swim-up fry, GPX activity was significantly higher in both  
36 Se-supplemented treatments with the highest activity in the SO group. Se-supplementation  
37 enhanced the expression of hepatic SelPa in broodstock males and females along with  
38 MsrB1, GPx1a, GPx4a2, CAT, Gclc, Keap1 and MsrB2 in male liver. In swim-up fry, only  
39 organic Se-supplementation led to a higher gene expression for SelPa, GPX1a, GPX1b2,  
40 CAT and MsrB2. An improvement of the oxidative status in whole-body of swim-up fry could  
41 not be confirmed in this study as the GSH/GSSG was even lower in swim-up fry of Se-  
42 supplemented groups, whereas the 8-isoprostane levels were lower in the SS group and no  
43 effect on protein carbonyl levels could be detected. Organic Se-supplementation led to a  
44 significant increase in the  $\alpha$ -tocopherol and vitamin C levels in the progeny. These results  
45 show that dietary Se in broodstock nutrition influences spawning occurrence and along with  
46 the parental transfer, several traits in the progeny are affected.

47

48

49 **Keywords:**

50 Rainbow trout; Broodstock; Progeny; Selenium; Antioxidant

51

## 52 **1. Introduction**

53 While fishmeal based diets naturally contain high amounts of selenium (Se) covering the  
54 nutritional requirements (Hilton et al., 1980), plant based diets are generally lower and vary  
55 more widely in their Se content (Combs, 2001). With the ongoing replacement of fish meal  
56 and fish oil in aquafeeds, there is a need to better understand the metabolic role of Se,  
57 which is complex and not yet fully explored. The Se requirements in fish are reported to vary  
58 between 0.15 and 0.70 mg/kg diet (Antony Jesu Prabhu et al., 2016), depending on species  
59 (Watanabe et al., 1997), life stage (Bell et al., 1985) and bioavailability of the Se form (Wang  
60 and Lovell, 1997; Penglase et al., 2014a). Thereby, organic Se forms have been described  
61 to have a higher bioavailability compared to inorganic forms, leading to increased tissue Se  
62 content (Le and Fotedar, 2014; Fontagné-Dicharry et al., 2015; Antony Jesu Prabhu et al.,  
63 2016). Dietary Se, both in surplus and deficiency, can have severe consequences for the  
64 fish. Already a dietary Se level of 3 mg/kg, slightly above the recommended level, can in the  
65 long term induce adverse effects (Berntssen et al., 2018). On the other hand, Se deficiency  
66 can reduce the expression and activity of selenoproteins (Hesketh, 2008; Elia et al., 2011;  
67 Penglase et al., 2014a), of which many are involved in the antioxidant protection of the cell  
68 (Papp et al., 2007). In a previous study, working with low Se levels in rainbow trout  
69 (*Oncorhynchus mykiss*) fry, the positive impact of Se supplementation on Se-dependent  
70 GPX activity and gene expression was demonstrated, especially when an organic Se  
71 supplement was used (Fontagné-Dicharry et al., 2015). Se supplementation was found to  
72 increase the expression of selenoprotein P not only in rainbow trout, but also in zebrafish  
73 (*Danio rerio*) (Penglase et al., 2014a; Fontagné-Dicharry et al., 2015; Pacitti et al., 2015;  
74 Wang et al., 2018). Under a dietary Se deficiency, besides the effects at the cellular level, a  
75 reduced growth and an increased mortality in fish are also reported (Wang et al., 2013). In  
76 rainbow trout, the negative impact of Se deficiency on body weight could not be detected  
77 (Bell et al., 1986), but liver vitamin E levels were significantly reduced and the combined  
78 effect of vitamin E and Se deficiency led to a reduced weight gain (Bell et al., 1985). Most of  
79 the studies investigating the effect of dietary Se on the metabolism in fish have been

80 undertaken with juveniles or early stages and there is little information on the effects of Se in  
81 broodstock nutrition, except studies investigating the impact of Se-supplementation at high  
82 levels (Hardy et al., 2010; Penglase et al., 2014a, 2014b). However, the reproduction period  
83 is substantially different to other life stages as energy is not used for growth, but mobilized to  
84 the reproductive organs; especially in females where nutrients are accumulated in the  
85 ovarian oocytes during vitellogenesis. Therefore, Se deficiency effects observed at early  
86 stages might be different in broodstock fish and might affect spawn quality. Male fertility and  
87 reproduction have been shown to be reduced in several animal species fed Se-deficient  
88 diets (Hosnedlova et al., 2017) and could be linked to the presence of the selenoprotein  
89 GPx4 in male sperm (Imai et al., 2009). A reduced antioxidant capacity of offspring by  
90 maternal Se deficiency have already been described in broilers (Pappas et al., 2006; Zhang  
91 et al., 2014; Xiao et al., 2016). Furthermore, the dietary supplementation of Se in poultry  
92 breeders led to an increased fertility of males and maternal transfer of Se, improving the  
93 oxidative status in the progeny (Słowińska et al., 2011, Wang et al., 2011; Zhang et al.,  
94 2014). Se toxicity trials undertaken with zebrafish (Thomas and Janz, 2015) and brown trout  
95 (*Salmo trutta*) (Covington et al., 2018) suggest that a parental transfer of Se in fish exists at  
96 toxic levels. The current study was undertaken to address whether similar effects are  
97 detected in broodstock fish fed nutritionally relevant dietary levels of Se in different forms.

98

## 99 **2. Material and Methods**

### 100 *2.1. Experimental set up*

101 Three-year-old rainbow trout (*Oncorhynchus mykiss*) females and males of the same genetic  
102 origin from the INRA experimental fish farm of Léés-Athas (Pyrénées-Atlantiques, France)  
103 were used as broodstock. Rainbow trout broodstock were randomly allocated to three  
104 circular 2-m diameter tanks supplied with flow-through (1L per sec) spring water at  $8 \pm 1$  °C  
105 with 25 females (initial mean weight:  $1.1 \pm 0.2$  kg, second reproduction season) and 15  
106 males ( $0.9 \pm 0.3$  kg) per tank (stocking density: 40 kg/m<sup>3</sup>). The fish were hand fed once a  
107 day to visual satiation for a period of 6 months prior to spawning. Each fish was tagged in

108 the dorsal muscle with a passive integrated transponder (PIT, 12 x 2 mm, ISO 11784/11785,  
109 IER, Suresnes, France) and the individual growth was followed until spawning. During the  
110 spawning period, fish were regularly checked for ovulation and at spawning, oocytes of each  
111 female were collected through stripping. At the first spawning date, one female from the SS-  
112 group and two females from the SO-group were over-mature (with oocytes exhibiting polar  
113 condensation of vitellus and lipid goblets) and they were excluded from the trial as well as  
114 one NC female during the second spawning date due to low spawning quantity and quality.  
115 Otherwise, two pools of 50 oocytes from each female were lined up to measure total length  
116 and afterwards weighed to calculate the average diameter and weight of oocytes,  
117 respectively. All other eggs were fertilized with sperm collected on the same day from a pool  
118 of males of the same dietary group. Eggs from each female were reared separately in small  
119 trays supplied with flow-through spring water at  $8 \pm 1$  °C to follow individual hatching data.  
120 The embryonic development was followed until swim-up fry stage. The survival was  
121 monitored and dead eggs were removed every two days. Survival rates were calculated at  
122 eyed stage (33 dpf), hatching (46 dpf) and swim-up stage (65 dpf).

123

## 124 2.2. Sample collection

125 Broodstock fish were sampled at spawning. First, fish were anaesthetized with benzocaine  
126 (30 mg/L) for stripping and afterwards killed by a sharp blow on the head for liver and muscle  
127 sampling. Muscle tissue was sampled in duplicate at the dorsal fin as approximately 15g of  
128 fillet. The weights of whole fish, spawn (total eggs collected from each female after stripping  
129 and removal of coelomic fluid) and liver were recorded to calculate absolute and relative  
130 fecundity (number of eggs/female and number of eggs/kg female, respectively), gonado-  
131 somatic index (GSI, percentage of spawn weight to weight of female) and hepatosomatic  
132 index (HSI, percentage of liver weight to weight of fish). Tissue samples and oocytes (2 x 50  
133 units per female) were immediately frozen in liquid nitrogen and stored at -80 °C until further  
134 analysis. The progeny was sampled at swim-up fry stage. Pooled fry, for every female  
135 separately, were euthanized with an overdose of benzocaine (60 mg/L), weighed and

136 afterwards subdivided in different sample size according to planned analysis, before freezing  
137 as whole-body fry in liquid nitrogen and stored at -80°C until further analysis.

138 All procedures were performed in compliance with the European Directive 2010/63/EU for  
139 the protection of animals used for scientific purposes and the French Decree no. 2013-118  
140 for animal experimentation.

141

### 142 *2.3. Experimental diets*

143 Diets were manufactured using a twin-screw extruder (BC 45, Clextral, Firminy, France) at  
144 the INRA experimental facilities in Donzacq (Landes, France). The three diets (NC, SS and  
145 SO) were based on plant-derived proteins and had similar levels of crude protein (50%) and  
146 total lipid (22%) and differed only in Se content (Table 1). The NC-diet containing 0.3 mg  
147 Se/kg feed was not supplemented with Se and served as negative control. The SS and SO  
148 diets were supplemented with either sodium selenite (SS-diet) or hydroxy-selenomethionine  
149 (Selisseo®, SO-diet). In both supplemented diets, the targeted Se concentration was 0.6 mg  
150 Se/kg, which remained lower to concentrations usually found in fish meal based diets  
151 (Fontagné-Dicharry et al., 2015), but feed analyses gave a final dietary Se content of 0.8 mg  
152 Se/kg in SS diet and 0.7 mg Se/kg in SO diet.

153

### 154 *2.4. Proximate, mineral and fatty acid analysis*

155 For proximate composition of diets, dry matter (DM) was determined after drying at 105 °C  
156 for 24h, protein (N × 6.25) by Kjeldahl method after acid digestion (ISO, 1984), ash after 10h  
157 incineration at 550 °C and gross energy in an adiabatic bomb calorimeter. Total lipid in diets  
158 and broodstock muscle was extracted and quantified gravimetrically following Folch et al.  
159 (1957), using dichloromethane instead of chloroform. Fatty acid methyl esters were prepared  
160 and analyzed as previously described (Fontagné et al., 2008). Total phosphorus was  
161 determined by the molybdate blue/ascorbic acid method at 820 nm after mineralization and  
162 acid digestion (AFNOR, 1992). Total Se was measured using inductively coupled plasma  
163 mass spectrometry (ICP-MS, Agilent series 7500cx) by Ultra Trace Analyses Aquitaine



164 (UT2A, Pau, France) for diets, broodstock tissues and oocytes according to Vacchina and  
165 Dumont (2018) and by the Institut des Sciences Analytiques et de Physico-Chimie pour  
166 l'Environnement et les Matériaux (IPREM, Pau, France) for swim-up fry as described by  
167 Godin et al. (2015). Selenomethionine (SeMet) and selenocysteine (SeCys) were analyzed  
168 by liquid chromatography (HPLC, Agilent series 1200) coupled to ICP-MS by UT2A for  
169 oocytes according to Vacchina et al. (2018) and by IPREM for swim-up fry according to  
170 Godin et al. (2015).

171

## 172 *2.5. Antioxidant enzyme activity and mRNA levels*

173 Antioxidant enzyme activity was measured as previously described by Fontagné-Dicharry et  
174 al. (2014) in 0.3 g samples of broodstock liver and pooled whole-body fry. Briefly, all  
175 samples were first homogenized with an Ultra-turrax in an eight-time dilution of 20 mM-  
176 phosphate buffer (pH 7.4) containing 1mM EDTA, afterwards centrifuged at 2000g for 10  
177 min, the supernatant diluted in 0.5% triton solution and incubated for one hour before using  
178 the different enzyme assays. The glutathione peroxidase (GPX, EC 1.11.1.9) activity was  
179 measured in a solution of 50 mM phosphate buffer (pH 7.4), 1 mM EDTA, 2 mM sodium  
180 azide, 2 mM GSH, 0.1 mM NADPH and 0.2 mM glutathione reductase (GR) following the  
181 reduction of cumene hydroperoxide (0.2 mM) for Se-dependent GPX (SeGPX) and H<sub>2</sub>O<sub>2</sub> (50  
182 μM) for total GPX (totGPX) at 30 °C and 340 nm. Superoxide dismutase (SOD, EC 1.15.1.1)  
183 activity was measured by monitoring the inhibition of nitrotetrazolium reduction at 550 nm,  
184 and was expressed as the amount of enzyme required to inhibit the rate of nitrotetrazolium  
185 reduction by 50% per mg protein at 37 °C. The catalase (CAT, EC 1.11.1.6) activity was  
186 measured by following the reduction of H<sub>2</sub>O<sub>2</sub> in a solution of 67 mM phosphate buffer, 1 mM  
187 EDTA and 20 mM H<sub>2</sub>O<sub>2</sub> (pH 7.4) at 30 °C and 240 nm. GR (EC 1.8.1.7) activity was  
188 determined measuring the reduction of NADPH in a solution containing 100 mM phosphate  
189 buffer (pH 7.5), 1 mM GSSG and 50 μM NADPH at 30 °C and 340 nm. Glutathione-S-  
190 transferase (GST, EC 2.5.1.18) activity was measured in a solution of 100 mM phosphate  
191 buffer (pH 6.5), 1 mM EDTA, 1 mM GSH and 1 mM CDNB at 30°C and 340nm, using the

192 extinction coefficient of  $9.61 \text{ mM}^{-1}/\text{cm}$ . One unit of enzyme is defined as the amount needed  
193 to catalyze the reaction of  $1 \text{ }\mu\text{M}$  substrate per minute. The protein concentration was  
194 determined according to Lowry et al. (1951), using bovine serum albumin (BSA) as a  
195 standard.

196 Total RNA was isolated from 100 mg samples of broodstock liver and a pool of three whole-  
197 body swim-up fry by using Trizol reagent (Invitrogen, Cergy-Pontoise, France). For  
198 quantitative RT-PCR, complementary DNA was generated from 1 mg total RNA using  
199 SuperScript III RT (Invitrogen) and a mix of oligo (dT)<sub>15</sub> and random primers (Promega,  
200 Charbonnières, France). For each sample, RT was performed in duplicate and quantitative  
201 PCR analyses were performed in LightCycler® 480 Instrument II (Roche Diagnostics,  
202 Meylan, France) using LightCycler® 480 SYBR Green I Master mix (Roche Diagnostics).  
203 Total reaction volume was  $6 \text{ }\mu\text{L}$ , with  $2 \text{ }\mu\text{L}$  of the diluted RT reaction mixture (dilution 40) and  
204  $4 \text{ }\mu\text{L}$  of master mix added with  $0.4 \text{ mM}$  of each primer (Table 2). The PCR protocol was  
205 initiated at  $95 \text{ }^\circ\text{C}$  for 10 min for initial denaturation of the cDNA and hot-start Taq-polymerase  
206 activation, followed by 45 cycles of a three-step amplification program (15 s at  $95 \text{ }^\circ\text{C}$ , 10 s at  
207 the melting temperature of  $60 \text{ }^\circ\text{C}$ , 4.8 s at  $72 \text{ }^\circ\text{C}$ ). Melting curves were systematically  
208 monitored (5 s at  $95 \text{ }^\circ\text{C}$ , 1 min at  $65 \text{ }^\circ\text{C}$ , temperature slope at  $0.11 \text{ }^\circ\text{C}/\text{s}$  from  $65$  to  $97 \text{ }^\circ\text{C}$ ) at  
209 the end of the last amplification cycle to confirm the specificity of the amplification reaction.  
210 Relative quantification of target gene transcripts was performed using  $\beta$ -actin as the  
211 reference gene and NC as the reference group using the  $\Delta\Delta\text{Ct}$  method (Pfaffl, 2001).

212

### 213 *2.6. Glutathione and oxidative status in the progeny*

214 Total (GSht), oxidized (GSSG) and reduced glutathione (GSH) were measured in  $0.3 \text{ g}$   
215 homogenized liver tissues and  $1 \text{ g}$  of pooled oocyte and fry samples using Cayman  
216 glutathione assay kit (Bertin Pharma, Montigny-le-Bretonneux, France) according to the  
217 manufacturer's instructions with protein concentration assessed by the method of Lowry et  
218 al. (1951) using BSA as a standard. Lipid peroxidation was assessed by measuring  
219 concentration of 8-isoprostanes (8-isoPT), the product of non-enzymatic peroxidation of

220 arachidonic acid by reactive oxygen species in 0.5 g of pooled fry as described by Fontagné  
221 et al. (2006) using Cayman Chemical 8-isoprostane enzyme immunoassay kit (Spi-Bio,  
222 Massy, France) according to the manufacturer's instructions. Protein oxidation was  
223 assessed using the spectrophotometric determination of protein carbonyls by derivatization  
224 with 2,4-dinitrophenylhydrazine (DNPH) at 370 nm according to Armenteros et al. (2009)  
225 with protein concentration calculated from absorption at 280 nm in the blank sample using  
226 bovine serum albumin (BSA) as standard. HPLC was used for determination of vitamin C  
227 according to the method described by Mæland and Waagbø (1998). Tocopherols were  
228 extracted from a pool of six oocytes and three whole-body fry according to Folch et al.  
229 (1957) using dichloromethane instead of chloroform. They were analyzed by HPLC on a  
230 Phenomenex Luna® PFP(2) column (100 Å, 3 µm, 100 x 2 mm), with an isocratic mobile  
231 phase of methanol/water (93:7 v/v) at a flow rate of 0.5 mL/min and column oven  
232 temperature of 40 °C using a Waters Alliance 2695 separation module with a Waters 2475  
233 multi wavelength fluorescence detector set at 295/330 nm (excitation/emission) according to  
234 Górnas et al. (2014). The concentration of tocopherol per individual was calculated using the  
235 average weight of oocyte or whole-body fry.

236

### 237 *2.7. Statistical analysis*

238 Results are given as mean ± standard error (SEM). Differences between dietary groups  
239 were analyzed in statistical software R (R Development Core Team, 2008) or SigmaStat 3  
240 (SPSS, Chicago, IL, USA) using one-way ANOVA. Tocopherol data were analyzed using  
241 two-way ANOVA to compare data from oocytes with data at swim-up fry stage. All data were  
242 tested for normal distribution and homogeneity and only in case the assumptions were not  
243 respected, the ANOVA was performed instead on ranks. The binary spawning data were  
244 analyzed pairwise using  $\chi^2$  test. Percentages were arc-sin transformed before analysis. A  
245 Newman-Keuls post-hoc test was used to compare means, when a significant difference ( $P$   
246 < 0.05) was found.

247

### 248 3. Results

#### 249 3.1. Growth and reproductive performance in broodstock

250 All three diets were readily accepted by rainbow trout broodstock. In males and females, no  
251 significant difference in initial or final body weight was detected between the three dietary  
252 groups (Table 3). In females, no significant difference in daily growth between the control  
253 group and the supplemented groups was found. However, the daily growth index was higher  
254 in females fed SO compared to SS. In the groups NC and SS, six males and in SO, one  
255 female and four males died during the feeding trial before spawning. HSI, muscle lipid  
256 content and muscle fatty acid profile (data not shown) were not significantly different  
257 between groups for both males and females. At the end of the spawning period in January,  
258 the total number of fish spawning was significantly higher in the two Se-supplemented  
259 groups SS and SO compared to the control group NC. Also, females fed the organic Se-  
260 supplemented diet SO began to spawn significantly earlier compared to females fed the  
261 inorganic Se-supplemented diet SS (Fig. 1). No significant difference in reproductive  
262 performance, including GSI, fecundity or egg size could be detected (Table 3). During  
263 embryonic development, there was no significant difference in survival between NC and the  
264 supplemented groups. However, the survival rate was significantly lower in the SS-group  
265 than in SO from hatching onwards.

266

#### 267 3.2. Se and its transfer from broodstock to progeny

268 Total Se content was significantly higher in muscle of females fed SO, compared to the ones  
269 fed NC or SS (Fig. 2A). The same tendency was noticed in female liver, but no significant  
270 difference between the three treatments was detected (Fig. 2B). Total Se content in milt was  
271 not significantly different between groups (Fig. 2C). On the other hand, total Se content in  
272 oocytes was significantly higher for females fed SO and lower in NC group compared to the  
273 SS treatment (Fig. 2D).

274 The majority of Se in oocytes was present in the form of SeMet, which was significantly  
275 higher in Se-supplemented groups compared to the control group NC with the highest

276 concentration in the organic Se treatment, while the SeCys content was not significantly  
277 different between the groups (Table 4). As for oocytes, the total Se content in swim-up fry  
278 before first-feeding was the lowest in NC, followed by SS and highest in SO (Fig. 2E).  
279 However, with smaller sample size for Se speciation analyses ( $n = 3$ ), no significant  
280 difference between the groups SS and SO were found (Table 4). SeCys content was not  
281 significantly different between the three dietary groups of swim-up fry. Only swim-up fry from  
282 the organic Se-supplemented group SO showed an increase in SeMet concentration  
283 compared to the two other groups (Table 4). The total Se content per individual decreased  
284 significantly from oocyte to swim-up fry stage in SS ( $18 \pm 2$  to  $15 \pm 1$  ng Se per individual)  
285 and SO ( $29 \pm 3$  to  $23 \pm 1$  ng Se per individual) groups whereas no significant change in Se  
286 content was noticed for NC ( $11 \pm 1$  ng Se per individual).

287

### 288 *3.3. Changes in selenoproteins and other antioxidant enzyme activity and mRNA levels in* 289 *broodstock and progeny*

290 There was no effect of broodstock nutrition on GPX (Fig. 3A and 3B) or other antioxidant  
291 enzyme activity in liver of female or male (Table 5). In whole-body swim-up fry, totGPX  
292 activity was significantly higher in both Se-supplemented treatments with the highest activity  
293 in the SO group (Fig. 3C). This was associated with higher SeGPX activity in SO-group  
294 compared to the two other groups. The activity of other antioxidant enzymes was not  
295 significantly affected by Se supplementation (Table 5).

296 The mRNA expression of selenoprotein genes including the different GPX genes was not  
297 significantly affected by dietary Se supplementation in broodstock female liver except for  
298 SelPa and SelPa2 that were higher expressed in liver of broodstock females fed the  
299 inorganic Se-supplemented diet SS compared to the two other groups (Fig. 4A). The other  
300 antioxidant genes not coding for selenoproteins were not significantly affected by dietary Se  
301 supplementation in female liver (Fig. 4B). In male liver, all the three selenoproteins P were  
302 found to be higher expressed in the Se-supplemented treatments SS and SO while GPx1a  
303 was significantly higher expressed only in the inorganic Se treatment SS and MsrB1 only in

304 the organic Se treatment SO (Fig. 5A). GPX4a2 mRNA levels were significantly higher in  
305 liver of broodstock male fed the organic Se-supplemented diet SO than in the inorganic Se-  
306 supplemented group SS. Regarding non-selenoprotein genes, CAT, Gclc and MsrB2 mRNA  
307 levels were significantly higher in liver of broodstock males fed the two Se-supplemented  
308 groups while Keap1 mRNA levels were only higher in male livers from the inorganic Se  
309 supplemented treatment SS compared to the control group NC (Fig. 5B). In whole-body  
310 swim-up fry, gene expression of the SO group with organic Se supplementation was  
311 enhanced not only for the two selenoproteins Pa and Pa2, but also for the glutathione  
312 peroxidases GPx1a and GPx1b2 (Fig. 6A). For other non-selenoproteins involved in  
313 antioxidant protection, no significant difference in mRNA levels could be detected between  
314 the swim-up fry groups except for CAT and MsrB2 that were higher expressed in the organic  
315 Se-supplemented group SO (Fig. 6B).

316

#### 317 *3.4. Glutathione levels in rainbow trout broodstock and offspring*

318 GSHt, GSSG and GSH contents in male or female liver were not significantly different  
319 between the three dietary groups (Fig. 7A and 7B). In whole-body swim-up fry, GSH was  
320 significantly lower in the organic Se-supplemented group SO, while GSSG was higher in  
321 both Se-supplemented groups compared to the control group NC (Fig. 7C). Glutathione  
322 content was also measured in oocytes, but GSSG was not detectable, while no significant  
323 difference in total glutathione was detected between the three dietary groups of oocytes  
324 ( $0.68 \pm 0.04 \mu\text{mol/mg protein}$ ).

325

#### 326 *3.5. Oxidative status in rainbow trout offspring*

327 At whole-body swim-up fry stage, the GSH/GSSG ratio was significantly lower in the  
328 offspring from broodstock fed the Se-supplemented diets SS and SO compared to the  
329 control group NC (Table 6). The concentration of 8-isoprostanes was significantly lower in  
330 whole-body swim-up fry in the inorganic Se treatment SS compared to the control group NC  
331 and organic Se-supplemented group SO. Parental Se-supplementation had no significant

332 effect on the concentration of protein carbonyls in whole-body swim-up fry. However, there  
333 was a tendency for lower protein carbonyl content in the organic Se treatment SO, indicative  
334 of an improved antioxidant status. This was confirmed with vitamin C that displayed  
335 significantly higher amounts in whole-body swim-up fry from the organic Se-supplemented  
336 SO treatment compared to the two other groups.

337

### 338 *3.6. Tocopherol content in rainbow trout offspring*

339 Organic Se supplementation in rainbow trout broodstock diet significantly increased the  $\alpha$ -  
340 tocopherol concentration per individual in the offspring. The concentrations of  $\gamma$ - and  $\delta$ -  
341 tocopherols were not significantly affected by dietary broodstock Se supplementation. A  
342 significant decrease between oocyte and swim-up fry stage was noticed for  $\delta$ -tocopherol  
343 (Table 7).

344

## 345 **4. Discussion**

346 The recent edition of NRC (2011) stated that micronutrient requirements for farmed fish and  
347 shrimp are another area where knowledge is lacking; whether dietary micronutrient  
348 requirements should be based on minimal levels required to prevent clinical deficiency signs  
349 or impaired growth or based on other criteria is an open question. Studies on the  
350 determination of nutrient requirements should rely on more than one response criterion to  
351 test the effect of increasing levels of a nutrient and eventually estimate minimal dietary  
352 inclusion levels for each criterion tested, whenever possible.

353

### 354 *4.1. Organic and inorganic Se supplementation affects spawning rate and timing*

355 We did not find any positive effect of Se-supplementation to a plant-ingredient based diet on  
356 the growth performance of rainbow trout broodstock. It could indicate that the Se levels used  
357 in the control diet did not provoke a severe deficiency situation, as was also shown, in an  
358 earlier study with rainbow trout, that a Se level of 0.025 mg Se/kg did not significantly affect  
359 growth rates (Bell et al., 1986). On the other hand, the growth performance in all treatments

360 of this experiment was low, which can be attributed in part to low water temperatures and the  
361 use of plant-based diets or it might be related to the increased demand of energy in  
362 reproductive organs during spawning period. These factors might have masked the impact of  
363 Se-supplementation on broodstock growth. We did not also find any significant effect of the  
364 form of Se-supplementation on reproductive performance parameters, including absolute  
365 and relative fecundity, GSI and egg size and weight. These results are in line with  
366 observations in zebrafish, where no significant difference in number of eggs and mating  
367 success could be found between groups fed deficient (0.09 mg Se/kg) vs. replete (0.65 mg  
368 Se /kg) diets (Penglase et al., 2014a). Also, similar to the mentioned zebrafish trial, the  
369 survival of the progeny was not significantly different between the control group and fish fed  
370 Se supplemented diets, however we found a slightly lower survival in the inorganic  
371 supplemented group compared to the control group. But even without a detectable effect on  
372 growth, the number of females spawning was significantly higher in both Se-supplemented  
373 groups and females fed organic Se in form of hydroxy-selenomethionine spawned  
374 significantly earlier compared to females receiving the sodium selenite diet. The low survival  
375 in progeny of selenite group can be traced back to given ascending females, spawning at the  
376 early spawning dates, while progeny of the majority of females in this group did not show  
377 any elevated mortality. The differences in spawning pattern and survival between the groups  
378 receiving different forms of dietary Se supplementation could be connected to modified  
379 vitellogenesis and blood steroid concentrations as these parameters were described to be  
380 affected in rainbow trout females fed dietary SeMet at a high level of 4.54 mg/kg (Wiseman  
381 et al., 2011). It might have been interesting to also analyze plasma vitellogenin levels in the  
382 present study.

383 The results indicate that a plant-protein based diet with a basal Se level of 0.3 ppm was not  
384 sufficiently deficient to affect growth of rainbow trout broodstock, but might negatively affect  
385 reproduction by decreasing the number of females spawning, which can be improved by  
386 supplementing Se, especially as organic form.

387



#### 388 4.2. Effective Se transfer from broodstock to progeny

389 Tissue analysis revealed elevated female liver and muscle tissue Se concentrations in  
390 supplemented groups, compared to the control group, and the concentration in fish fed diets  
391 with organic Se supplementation were even higher compared to fish fed inorganic Se diets.  
392 The increase of Se in muscle tissue of fish fed Se-supplemented diets was also recently  
393 reported in Atlantic salmon (*Salmo salar*), finding higher Se retention for organic Se  
394 supplementation in the form of SeMet compared to inorganic supplementation (Sele et al.,  
395 2018). The higher retention for organic Se (SeMet), compared to inorganic Se (selenite) was  
396 previously described also in rainbow trout fry (Rider et al., 2009; Godin et al., 2015).  
397 However, this effect is not systematic, as in some other studies, no effect on tissue Se  
398 accumulation was described, when working with similar amounts of Se as studied here (Han  
399 et al., 2011). We found elevated Se concentrations in the progeny at oocyte and swim-up fry  
400 stage before first feeding in Se-supplemented groups with higher transfer in the group  
401 supplemented with hydroxy-selenomethionine. These results are in line with results obtained  
402 in hens that describe the parental transfer of Se to the progeny (Pappas et al., 2006; Wang  
403 et al., 2011). The Se speciation in oocytes and swim-up fry could show that the SeMet can  
404 be found in tissues in higher concentrations compared to SeCys. Thereby, Se  
405 supplementation increased the SeMet levels, while SeCys was not affected. The elevated  
406 levels of SeMet in oocytes of the selenite treatment are surprising, especially as they are not  
407 found any more at swim-up fry stage. Indeed selenite in the Se metabolism is known to be  
408 metabolized to selenide and not transformed to SeMet (Burk and Hill, 2015). The elevated  
409 status of SeMet in the oocytes of the inorganic Se supplemented group found in this study  
410 might be linked to a transfer of SeMet from broodstock to the progeny, a process which  
411 could have been limited in the control group compared to the Se-supplemented groups. In a  
412 study with rainbow trout fry fed a diet supplemented with sodium selenite or SeMet, SeMet  
413 was found to be higher in the SeMet treatment compared to control group and selenite  
414 treatment, whereas in comparison to SeMet, the SeCys concentration was not affected by  
415 the supplementation at all (Godin et al., 2015). However, there were huge differences in the

416 amount of Se identified as SeMet and SeCys between the two tissues analyzed in this study,  
417 which could be also connected to different extraction methods used. In this case, the  
418 absolute concentration values should be considered carefully and preferably trends at each  
419 stage regarded. However, it cannot be excluded that other seleno-components are present  
420 in fry as other Se-containing peaks were observed in chromatograms.

421 These results showed that Se-supplementation to diets with low basal levels could increase  
422 the tissue Se concentration and that there was a transfer of Se from the broodstock to the  
423 progeny. The bioavailability and transfer of Se was stronger in broodstock fed hydroxy-  
424 selenomethionine compared to selenite.

425

#### 426 *4.3. An altered antioxidant metabolism in deficient Se broodstock and offspring* 427 *compensated by Se supplementation especially in organic form*

428 In animals, selenoprotein P is a selenoprotein often found to be regulated by Se level (Yuan  
429 et al., 2013; Penglase et al., 2014a; Fontagné-Dicharry et al., 2015) and this was confirmed  
430 in the present study where the SelPa expression was significantly lower in the control group.  
431 In hepatic tissue of both males and females, the strongest effect was found in the group fed  
432 inorganic Se supplemented diets, while in the whole-body swim-up fry, the effect is  
433 detectable only in the organic Se supplemented groups. In humans, Steinbrenner et al.  
434 (2006) found that hepatocyte-derived selenoprotein P concentrations can stimulate the  
435 GPX1 expression and activity in astrocytes. Similar to selenoprotein P, GPX1 mRNA levels  
436 and activity are often described to decrease in mammals under Se deficiency (Chow and  
437 Tappel, 1974; Sunde et al., 2009) and have been found to be a good biomarker of Se status  
438 in fish (Bell et al., 1985; Penglase et al., 2014a; Fontagné-Dicharry et al., 2015; Pacitti et al.,  
439 2015). Likewise, the present results showed a lower GPX1a expression in male liver of the  
440 control group, compared to the inorganic Se treatment, but there was no effect in female  
441 liver or GPX activity in broodstock male or female liver. In whole-body swim-up fry, GPX1a1  
442 and GPX1b2 expression, in correlation with GPX activity was increased in fry coming from  
443 broodstock fed the organic Se supplemented diet. In male liver, also GPX4a2 was influenced

444 by dietary Se supplementation with significantly higher levels in the organic Se  
445 supplemented treatment, compared to the group fed inorganic Se, while in a study with  
446 rainbow trout fry GPX4a1 was found to be the most sensitive isoform in terms of low Se  
447 levels (Fontagné-Dicharry et al., 2015). Se deficiency was described to lead to decreased  
448 levels of MsrB protein activity and mRNA expression in various tissues (Moskovitz and  
449 Stadtman, 2003). In the present study, the selenoprotein MsrB1 was higher expressed only  
450 in male liver of the group fed organic Se-supplemented diets, while the non-selenoprotein  
451 MsrB2 was regulated by dietary Se not only in male liver of both supplemented groups, but  
452 also in the progeny of the group fed organic selenium. In comparison to mice, where the  
453 selenoprotein MsrB1 is the most dominant Msr in liver and affected by dietary Se levels  
454 (Novoselov et al., 2010), these results in rainbow trout could indicate a different MsrB  
455 hierarchy in fish as MsrB2 was more sensitive to low dietary Se levels than MsrB1.  
456 These results showed that parental Se nutrition, especially supplementation with hydroxy-  
457 selenomethionine as an organic source of Se, can enhance not only the expression of  
458 proteins involved in antioxidant defense, such as SeIP, GPX and MsrB2, but can also  
459 increase the activity of GPX in the progeny, while the expression of some hepatic proteins in  
460 the broodstock was more strongly affected even by a supplementation with selenite.

461

#### 462 *4.4. Contrasted oxidative status in progeny related to Se broodstock nutrition*

463 As an integral part of several glutathione peroxidases, Se plays an important role in  
464 metabolizing hydrogen peroxides and lipid hydroperoxides into water and lipid alcohols  
465 (Brigelius-Flohé, 1999). This function is complementary to that of vitamin E as a lipid-soluble  
466 antioxidant and both are functioning in close link (Hamre, 2011). It has been shown that a  
467 combined deficiency of vitamin E and Se can lead to severe consequences, including  
468 suppressed growth, muscular dystrophy, exudative diathesis and death in fish (Poston et al.,  
469 1976; Gatlin et al., 1986; Watanabe et al., 1997), while Se-deficiency symptoms appear less  
470 severe, if adequate levels of vitamin E are provided (Bell et al., 1985, 1986). In the present  
471 study, vitamin E requirements were assumed to be fulfilled as per the NRC (2011) with more

472 than 50 mg tocopherol per kg diet. As organic Se supplementation led to an increase in  $\alpha$ -  
473 tocopherol levels in the progeny, this possibly indicates a Se deficiency or reduced Se  
474 availability in the control group and in the group receiving sodium selenite. Indeed, Se-  
475 deficiency has been reported to cause a reduction of vitamin E levels (Hamre, 2011). These  
476 results are in line with an earlier study in hens, describing significant increase in yolk vitamin  
477 E concentration by the supplementation of hydroxy-selenomethionine (Tufarelli et al., 2016).  
478 Elevated vitamin E and Se levels in the progeny of supplemented groups thus should  
479 provide them a better protection against oxidative damage. This was supported also by an  
480 elevated vitamin C level in organic Se-supplemented group in this study, as was also  
481 reported by Kumar et al. (2018) in striped catfish. However, protein carbonyls as an indicator  
482 of protein oxidation were not significantly affected by Se-supplementation in the present  
483 study, even though there was a tendency for lower protein carbonyl levels in the group with  
484 organic Se-supplementation. An effect might have been found at the single tissue level as in  
485 mice, where fewer protein carbonyls were found in isolated liver, kidney, cerebrum and  
486 cerebellum, when fed higher Se levels (Moskovitz and Stadtman, 2003). A closer look on 8-  
487 isoprostane levels showed a decrease only in the group with sodium selenite  
488 supplementation. This result is surprising as Küçükbay et al. (2009) found that, both  
489 inorganic and organic Se supplementation led to a linear decrease of both serum 8-  
490 isoprostane and malondialdehyde (MDA) levels. Similarly, in common carp, selenite  
491 supplementation did not even affect MDA levels, which were only decreased by feeding  
492 SeMet or nano-Se (Saffari et al., 2017). As 8-Isoprostanes are products of fatty acid  
493 (arachidonic acid) peroxidation, a more indirect effect of Se could be suspected in the  
494 present study as dietary Se has been shown to affect fatty acid composition in a previous  
495 study (Fontagné-Dicharry et al., 2015). Also, the GSH/GSSG ratio in the whole-body swim-  
496 up fry was found to be lower in supplemented groups compared to the control group, which  
497 is accompanied by an absence of effect on the mRNA levels of both glutamate-cysteine  
498 ligase catalytic subunit (Gclc), needed for the synthesis of glutathione, and glutathione  
499 reductase (GR), needed for the reduction of glutathione, while the GPX activity was higher in

500 supplemented groups. In more developed fry fed with different Se level, not only the GPX  
501 activity, but also the GSH/GSSG ratio was higher in groups fed Se-supplemented diets  
502 (Fontagné-Dicharry et al., 2015). The different results might be connected to differences in  
503 the glutathione metabolism, which seems to be boosting at swim-up fry stage as levels of  
504 total glutathione increased about ten times compared to oocytes and oxidized glutathione  
505 levels only became detectable at swim-up fry stage in this study. The indirect effect of  
506 parental feeding on glutathione status in the progeny will need further investigation.

507 The absence of significant effects on some markers of oxidative status, especially linked to  
508 glutathione metabolism, may indicate that even with the clear difference in Se transfer from  
509 broodstock to the progeny, the Se deficiency in the non-supplemented group was not critical  
510 enough to provoke a strong oxidative stress. Otherwise, the Se deficiency in broodstock  
511 might induce other metabolic effects leading to changes in oxidative status such as  
512 modification of one-carbon metabolism as suggested by Speckmann et al. (2017) in mice.

513

## 514 **5. Conclusion**

515 The present results demonstrate that dietary Se-supplementation in plant-ingredient based  
516 diets with low basal selenium levels can enhance the total number of females spawning,  
517 while hydroxy-selenomethionine supplementation even led to an earlier spawning. It was  
518 demonstrated for the first time a parental transfer of Se in the progeny with the higher effect  
519 observed with the better bioavailability of organic Se. In the progeny before first feeding, the  
520 high Se levels led to an increase in the activity and mRNA expression of GPX and other  
521 proteins involved in antioxidant protection, along with elevated vitamin E and C levels. The  
522 impact of Se broodstock nutrition on glutathione metabolism in the progeny is intriguing and  
523 requires further investigation. Further work with juvenile fish at later developmental stages is  
524 warranted to show, if the parental effects persist after exogenous feeding of the progeny.

525

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719 maternal selenium affect selenium retention, antioxidant status, and meat quality of  
720 56-day-old offspring of broiler breeders. *Poult. Sci.* 93, 2210–2219.
- 721

722 Table 1. Formulation and composition of experimental diets (g/100g dry weight).

Diet	NC	SS	SO
<i>Ingredients</i>			
Plant meals <sup>1</sup>	74	74	74
Crystalline amino acids and attractant mixture <sup>2</sup>	3.14	3.14	3.14
Soybean lecithin <sup>3</sup>	2	2	2
Fish oil <sup>3</sup>	8	8	8
Vegetable oils <sup>4</sup>	8	8	8
Carophyll® pink <sup>5</sup>	0.04	0.04	0.04
Vitamin and mineral mixture without Se <sup>6</sup>	4.82	4.82	4.82
Sodium selenite (µg/g diet) <sup>7</sup>	-	0.7	-
Selisseo® (µg/g diet) <sup>7</sup>	-	-	15
<i>Analytical composition</i>			
Dry matter (DM, %)	96	98	97
Crude protein (% DM)	49	50	50
Total lipid (% DM)	23	22	23
Gross energy (kJ/g DM)	25	25	25
Ash (% DM)	6	6	6
Phosphorus (% DM)	1.2	1.1	1.2
Se (mg/kg)	0.3	0.8	0.7
<i>Fatty acid profile (% total fatty acids)</i>			
14:0	5.1	4.6	5.0
16:0	25.5	24.4	25.6
18:0	3.1	3.2	3.3
Total saturates <sup>8</sup>	35.1	33.6	35.3
16:1	3.8	3.5	3.7
18:1	28.0	28.8	27.5
Total monoenes <sup>8</sup>	32.5	33.0	31.8
18:2 n-6	16.7	17.3	17.0
20:4 n-6	0.5	0.4	0.5
Total n-6	17.2	17.7	17.4
18:3 n-3	9.9	10.1	9.7
18:4 n-3	0.6	0.5	0.5
20:5 n-3	2.3	2.1	2.2
22:6 n-3	1.5	1.8	2.1
Total n-3	14.3	14.6	14.6

723 <sup>1</sup>Plant meals (% diet): 20% wheat gluten (Roquette), 18% corn gluten meal (Inzo), 15%  
724 soybean protein concentrate Estril®75 (Sopropêche), 6% soybean meal (Sud-Ouest  
725 Aliment), 5% rapeseed meal 00 (Sud-Ouest Aliment), 5% white lupin meal Farilup 500  
726 (Terrena), 3% dehulled pea meal Primatex (Sotexpro), 2% whole wheat (Sud-Ouest  
727 Aliment).

728 <sup>2</sup>Crystalline amino acids and attractant mixture (% diet): 1.34% L-lysine, 0.3% DL-  
729 methionine, 0.5 % glucosamine, 0.3% taurine, 0.3% betaine, 0.2% glycine, 0.2% alanine.

730 <sup>3</sup>Soybean lecithin from Louis François and fish oil from Sopropêche.

731 <sup>4</sup>Vegetable oils (% diet): 4% rapeseed oil, 2.4% linseed oil, 1.6% palm oil (Daudry).

732 <sup>5</sup>Contained 10% astaxanthin (DSM).

733 <sup>6</sup>Vitamin and mineral mixture without Se (per kg diet): retinol acetate, 55,000 IU;  
734 cholecalciferol, 2,500 IU; DL- $\alpha$ -tocopherol acetate, 50 IU; sodium menadione bisulfate, 10  
735 mg; thiamin-HCl, 1 mg; riboflavin, 4 mg; niacin, 10 mg; D-calcium pantothenate, 20 mg;  
736 pyridoxine-HCl, 3 mg; D-biotin, 0.2 mg; folic acid, 1 mg; cyanocobalamin, 10 µg; L-ascorbyl-  
737 2-polyphosphate, 50 mg; myo-inositol, 0.3 g; choline, 1 g; CaHPO<sub>4</sub>·2H<sub>2</sub>O, 33 g; CaCO<sub>3</sub>, 2.15  
738 g; Mg(OH)<sub>2</sub>, 1.24 g; KCl, 0.9 g; NaCl, 0.4 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.2 g; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 40 mg;

739  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 30 mg;  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 30 mg; NaF, 10 mg; KI, 0.4 mg;  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.2 mg. All  
740 ingredients were diluted with  $\alpha$ -cellulose.

741 <sup>7</sup>Sodium selenite contained 42% Se (Sigma-Aldrich) and Selisseo contained 2% Se  
742 (Adisseo).

743 <sup>8</sup>Total saturates include 12:0, 15:0, 17:0 and 20:0 and total monoenes include 14:1 and 20:1.

744 Table 2. Oligonucleotide primers used to assay mRNA levels by real-time quantitative RT-  
745 PCR.

Gene	Accession no.	Forward primer sequence	Reverse primer sequence	Amplicon size
SeIPa	HF969249.1	cagccacctggttgagtat	cctggagttagggccacca	82
SeIPa2	MH085054.1	accttgctgagccagaaact	cagacgaccacacctgtcat	129
SeIPb	HF969250.2	gtgtcgttcctcatcgtgaa	ggaacgtcagctcccacat	187
GPX1a	HE687021	aatgtggcgtcactctgagg	caattctcctgatggccaaa	131
GPX1b1	CA357669.1	cgagctccatgaacggtagc	tgctcccgttcacatccac	183
GPX1b2	HE687023	tcggacatcaggagaactgc	tcctccattcacatccac	121
GPX4a1	HE687024	gaaaggctcctgggaaatg	ctccaccacactgggatcat	112
GPX4a2	HE687025	agaaatacagggcgacggt	gcatctccgcaaactgagag	90
GPX4b	CA344428.1	ttggaggtcaggagccaggt	acccttccctgggctgtt	152
TR	HF969247.1	acaaaatcaaggcgaccaac	ggcagagagaacaggtcgtc	148
MsrB1	BX313019.3	ctggcctgccttactgaga	ttccacatcggacctgaa	87
MsrB2	BX311214.3	aggggacagagatgcccttc	cccatgagcctcttgaacg	149
MsrB3	CX041708.1	tcaggttggccttcttcta	cgaggtgagaaccacactga	118
MsrA	BT073595.1	agatggctatgcttgggatg	acctgtgcaggtctcttctg	137
SOD1	AF469663.1	tggtcctgtaagctgattg	ttgtcagctcctgcagtcac	201
SOD2	CA352127.1	tcctgacctgacctacgac	ggcctcctcattaaacctc	201
CAT	BX087110.3	tgatgtcacacaggtagcgta	gtgggctcagtggtgtgag	195
GR	HF969248.1	ctaagcgcagcgtcatagtg	acaccctgtctgacgacat	108
Gclc	GSONMT00065033001	caaccaactggcagacaatg	ccttgacaaggggatgaga	189
GST $\pi$	BX302932.3	tcgctgactggacgaaagga	cgaaggtcctcaacgccatc	196
Nrf2	CA360709.1	tgagctgcagcaatgtctga	ggtgggcaatgggtagaagc	124
Keap1	GSONMT00034445001	gctacgtgatgtctgccct	ggtacctcatagcggccagt	116
Keap1X	GSONMT00019673001	actgggaggttatgacggga	cctccgtcctctcatgcttc	179
NF- $\kappa$ B	BX880658.3	cagcgtcctaccaggctaaagagat	gctgttcgatccatccgactat	181
I $\kappa$ B $\alpha$	BT074199.1	agagacagactgcgtccac	cggcctcagtagcctctct	72
$\beta$ -Actin	AJ438158.1	gatgggccagaaagacagcta	tcgtcccagttggtgacgat	105

746 SeIP, selenoprotein P; GPX1, glutathione peroxidase 1 ; GPX4, glutathione peroxidase 4;  
747 TR, thioredoxin reductase; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2;  
748 CAT, catalase; GR, glutathione reductase; Gclc, glutamate-cysteine ligase catalytic subunit;  
749 GST $\pi$ , glutathione S-transferase  $\pi$ ; Nrf2, nuclear factor erythroid-2 related factor 2; Keap1,  
750 Kelch-like ECH-associated protein 1; NF- $\kappa$ B, nuclear factor kappa-light chain-enhancer of  
751 activated B cells; I $\kappa$ B $\alpha$ , NF- $\kappa$ B inhibitor  $\alpha$ .  
752

753 Table 3. Growth, spawning parameters and offspring performance of rainbow trout  
 754 broodstock fed different Se sources over 6 months at a constant water temperature of  $8 \pm$   
 755  $1^\circ\text{C}$ .

Diet	NC	SS	SO
<i>Male performance</i>			
Initial body weight (kg, n=15)	0.88 $\pm$ 0.06	0.90 $\pm$ 0.09	0.82 $\pm$ 0.07
Body weight after 6 month (kg, n=9-11)	1.20 $\pm$ 0.12	1.04 $\pm$ 0.09	1.03 $\pm$ 0.13
DGI <sup>1</sup> (% , n=9-11)	0.34 $\pm$ 0.08	0.28 $\pm$ 0.14	0.29 $\pm$ 0.12
HSI <sup>1</sup> (% , n=5)	1.1 $\pm$ 0.1	1.1 $\pm$ 1.1	1.5 $\pm$ 0.2
Muscle lipid content (% , n=5)	6.8 $\pm$ 0.8	6.7 $\pm$ 1.1	6.3 $\pm$ 0.6
<i>Female performance</i>			
Initial mean body weight (kg, n=25)	1.13 $\pm$ 0.05	1.11 $\pm$ 0.03	1.10 $\pm$ 0.04
Final mean body weight (kg, n=24-25)	1.30 $\pm$ 0.07	1.21 $\pm$ 0.04	1.32 $\pm$ 0.05
DGI <sup>1</sup> (% , n=10-21)	0.27 $\pm$ 0.05 <sup>ab</sup>	0.17 $\pm$ 0.04 <sup>b</sup>	0.32 $\pm$ 0.05 <sup>a</sup>
HSI <sup>1</sup> (% , n=10-21)	1.1 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.2
Muscle lipid content (% , n=8-18)	7.9 $\pm$ 0.6	6.2 $\pm$ 0.5	6.9 $\pm$ 0.4
GSI <sup>1</sup> (% , n=13-24)	15.6 $\pm$ 1.1	14.3 $\pm$ 1.0	15.6 $\pm$ 0.7
Spawn weight (g, n=13-24)	220 $\pm$ 16	168 $\pm$ 12	205 $\pm$ 13
Absolute fecundity (eggs/female, n=13-24)	3378 $\pm$ 209	2687 $\pm$ 266	3119 $\pm$ 136
Relative fecundity (eggs/kg female, n=13-24)	2381 $\pm$ 148	2269 $\pm$ 213	2418 $\pm$ 114
<i>Offspring performance</i>			
Egg diameter (mm, n=13-24)	4.1 $\pm$ 0.1	3.9 $\pm$ 0.1	4.1 $\pm$ 0.0
Egg mean weight (mg, n=13-24)	54 $\pm$ 3	50 $\pm$ 2	54 $\pm$ 1
Survival at eyed stage (% , n=12-21)	91 $\pm$ 2	79 $\pm$ 7	94 $\pm$ 1
Hatching rate (% , n=12-21)	89 $\pm$ 2 <sup>ab</sup>	76 $\pm$ 6 <sup>b</sup>	92 $\pm$ 1 <sup>a</sup>
Survival at swim-up fry stage (% , n=12-21)	87 $\pm$ 2 <sup>ab</sup>	75 $\pm$ 6 <sup>b</sup>	91 $\pm$ 1 <sup>a</sup>
Mean weight of swim-up fry (mg, n=12-21)	85 $\pm$ 3	82 $\pm$ 3	85 $\pm$ 2

756 Values are means  $\pm$  SEM. Within rows, means not sharing a common superscript letter are  
 757 significantly different ( $P < 0.05$ ) according to one-way ANOVA followed by a Newman-Keuls  
 758 test.

759 <sup>1</sup>DGI, daily growth index =  $100 \times (\text{final body weight}^{1/3} - \text{initial body weight}^{1/3})/\text{duration}$  (182  
 760 days); HSI, hepato-somatic index =  $100 \times (\text{wet liver weight}/\text{fish weight})$ ; GSI, gonado-  
 761 somatic index =  $100 \times (\text{wet spawn weight}/\text{female weight})$ ; Spawn weight = total egg weight  
 762 per female after stripping and removal of coelomic fluid; absolute fecundity = egg number per  
 763 female; relative fecundity = egg number/kg female.

764

765 Table 4. Total Se, SeMet and SeCys concentrations ( $\mu\text{g Se/kg wet weight}$ ), ratio of SeMet  
 766 and SeCys over total Se, SeMet to SeCys ratio and percentage of total Se in oocytes and  
 767 whole-body swim-up fry

	Setot	SeMet	SeCys	SeMet/Setot	SeCys/Setot	SeMet/SeCys	%Setot
<i>Oocytes</i>							
NC	214 $\pm$ 12 <sup>c</sup>	118 $\pm$ 18 <sup>c</sup>	89 $\pm$ 8	0.55 $\pm$ 0.06	0.42 $\pm$ 0.04 <sup>a</sup>	1.35 $\pm$ 0.25 <sup>b</sup>	97 $\pm$ 3
SS	321 $\pm$ 14 <sup>b</sup>	220 $\pm$ 24 <sup>b</sup>	102 $\pm$ 14	0.69 $\pm$ 0.06	0.32 $\pm$ 0.03 <sup>ab</sup>	2.24 $\pm$ 0.38 <sup>ab</sup>	100 $\pm$ 3
SO	521 $\pm$ 19 <sup>a</sup>	373 $\pm$ 5 <sup>a</sup>	116 $\pm$ 19	0.72 $\pm$ 0.02	0.22 $\pm$ 0.03 <sup>b</sup>	3.40 $\pm$ 0.56 <sup>a</sup>	94 $\pm$ 1
<i>Swim-up fry</i>							
NC	197 $\pm$ 17 <sup>b</sup>	47 $\pm$ 9 <sup>b</sup>	13 $\pm$ 2	0.24 $\pm$ 0.06	0.07 $\pm$ 0.01	3.68 $\pm$ 0.77 <sup>b</sup>	31 $\pm$ 6
SS	276 $\pm$ 11 <sup>a</sup>	40 $\pm$ 5 <sup>b</sup>	13 $\pm$ 1	0.15 $\pm$ 0.03	0.05 $\pm$ 0.01	2.95 $\pm$ 0.03 <sup>b</sup>	19 $\pm$ 4
SO	312 $\pm$ 10 <sup>a</sup>	84 $\pm$ 6 <sup>a</sup>	12 $\pm$ 2	0.27 $\pm$ 0.01	0.04 $\pm$ 0.00	6.89 $\pm$ 0.78 <sup>a</sup>	31 $\pm$ 1

768 Values are mean  $\pm$  SEM (n = 3). Within tissue and column values not sharing a common  
 769 superscript letter are significantly different ( $P < 0.05$ ) according to one-way ANOVA followed  
 770 by Newman-Keuls post-hoc test.

771

772 Table 5. Antioxidant enzyme activity in liver and whole-body swim-up fry from rainbow trout  
 773 fed different Se sources over 6 months at a constant water temperature of  $8 \pm 1^\circ\text{C}$ .

	<u>Female liver</u>			<u>Male liver</u>			<u>Swim-up fry</u>		
	NC	SS	SO	NC	SS	SO	NC	SS	SO
<i>Enzyme activity</i>									
SOD <sup>1</sup>	14 ± 1	14 ± 1	14 ± 1	19 ± 2	24 ± 2	18 ± 2	5.0 ± 0.2	4.9 ± 0.2	5.1 ± 0.3
CAT <sup>1</sup>	409 ± 32	373 ± 45	391 ± 50	502 ± 60	574 ± 52	424 ± 49	11 ± 1	11 ± 1	11 ± 1
GR <sup>2</sup>	8 ± 1	9 ± 1	9 ± 1	12 ± 2	7 ± 1	11 ± 1	8 ± 1	8 ± 1	8 ± 1
GST <sup>2</sup>	145 ± 10	154 ± 22	145 ± 17	232 ± 19	229 ± 35	220 ± 33	54 ± 4	57 ± 3	55 ± 4

774 Mean values ± SEM (n = 8, except for male liver: n = 5).. Within rows and for each tissue,  
 775 means not sharing a common superscript letter are significant different ( $P < 0.05$ ) according  
 776 to one-way ANOVA followed by Newman-Keuls post-hoc test.

777 <sup>1</sup>Expressed in U/mg protein

778 <sup>2</sup>Expressed in mU/mg protein



779 Table 6. Oxidative status assessed by GSH/GSSG ratio, 8-Isoprostanes, protein carbonyls  
 780 content and vitamin C in whole-body swim-up fry from rainbow trout broodstock fed different  
 781 Se sources over 6 months at a constant temperature of  $8 \pm 1^\circ\text{C}$ .

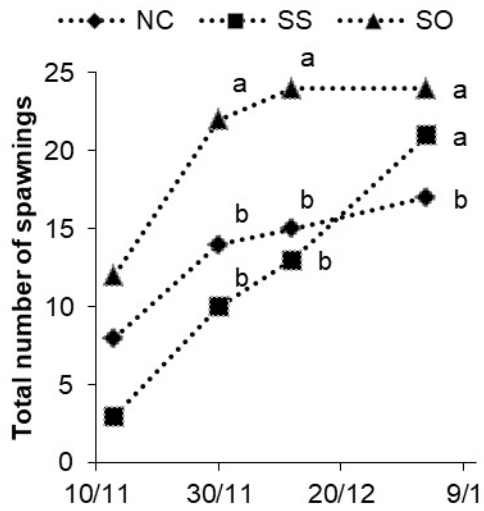
	NC	SS	SO
GSH:GSSG	$3.7 \pm 0.3^a$	$2.7 \pm 0.2^b$	$2.4 \pm 0.1^b$
8-Isoprostanes (pg/g)	$44 \pm 2^a$	$35 \pm 3^b$	$50 \pm 5^a$
Protein carbonyls (nmol/mg protein)	$19 \pm 1$	$19 \pm 1$	$16 \pm 1$
Vitamin C (mg/kg)	$29 \pm 1^b$	$30 \pm 2^b$	$37 \pm 2^a$

782 Within rows means  $\pm$  SEM (n = 8) not sharing a common superscript letter are significantly  
 783 different ( $P < 0.05$ ) according to one-way ANOVA followed by Newman-Kauls post-hoc test.  
 784

785 Table 7. Tocopherol content in offspring (oocyte and whole-body swim-up fry) from rainbow  
 786 trout broodstock fed different Se sources over 6 months at a constant temperature of  $8 \pm$   
 787  $1^\circ\text{C}$ .

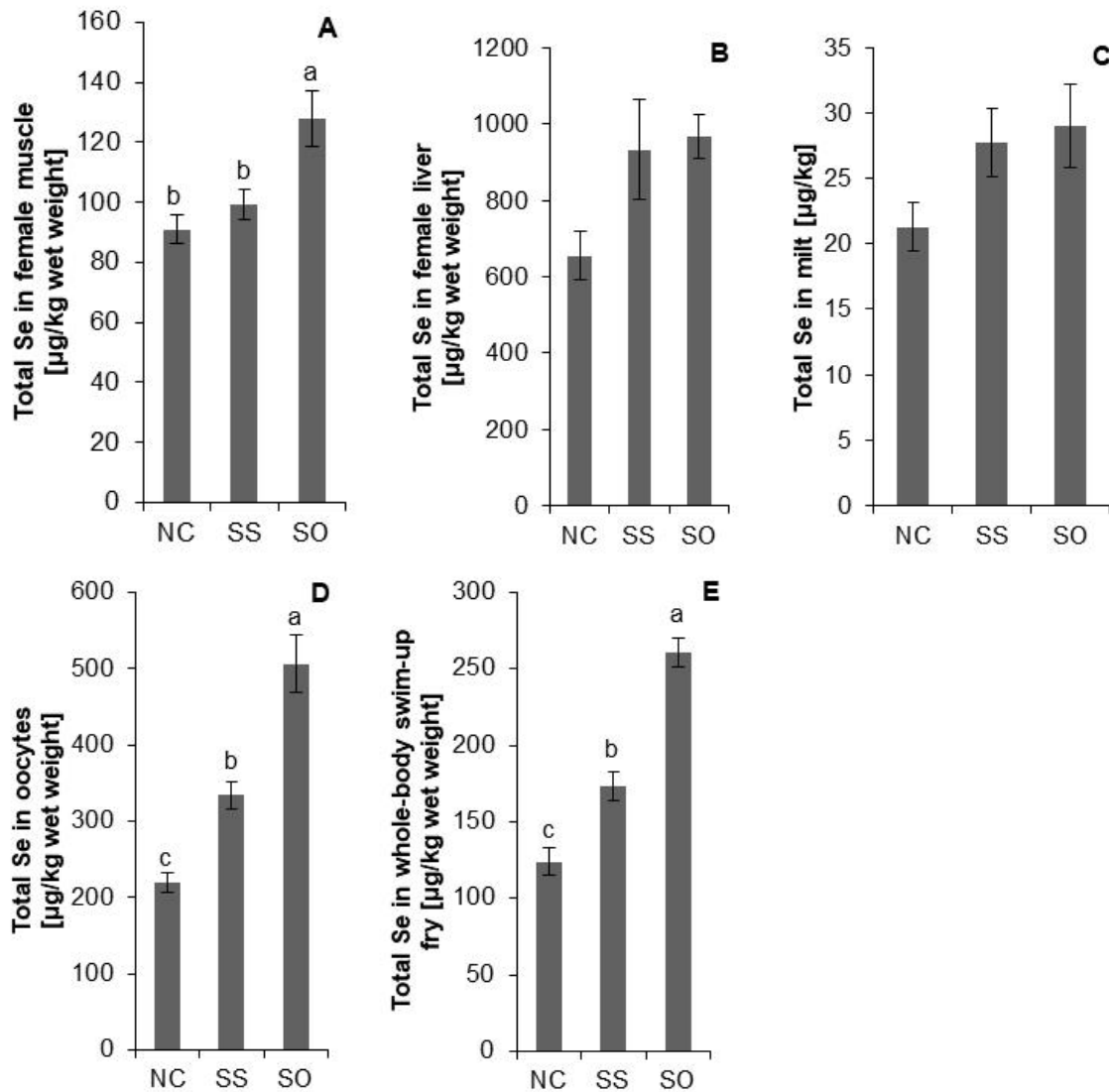
	<u>Dietary group</u>			<u>Stage</u>		<u>p-value</u>		
	NC	SS	SO	oocyte	swim-up fry	Diet	Stage	DxS
$\alpha$ -tocopherol	$1815 \pm 146^b$	$2056 \pm 176^{ab}$	$2472 \pm 168^a$	$2157 \pm 154$	$2071 \pm 132$	0.043	0.773	0.794
$\gamma$ -tocopherol	$254 \pm 23$	$249 \pm 26$	$254 \pm 15$	$263 \pm 17$	$241 \pm 18$	0.348	0.426	0.400
$\delta$ -tocopherol	$5.15 \pm 0.68$	$5.67 \pm 0.69$	$4.68 \pm 0.66$	$6.31 \pm 0.36^a$	$4.02 \pm 0.60^b$	0.967	<0.001	0.613

788 Values expressed in ng tocopherol per individual are means  $\pm$  SEM calculated from samples  
 789 of offspring ( $n = 8$  per dietary group and stage). Within rows and within dietary groups ( $n =$   
 790  $16$ ) and developmental stages ( $n = 24$ ), means not sharing a common superscript letter are  
 791 significantly different ( $P < 0.05$ ) according to two-way ANOVA followed by a Newman-Keuls  
 792 test.  
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**Fig. 1.** Total numbers of females spawning in rainbow trout broodstock fed different Se sources over a six months period prior to spawning at a constant water temperature of  $8 \pm 1^\circ\text{C}$ . For each date, total numbers of females spawning, not sharing the same superscript letter, are significantly different ( $P < 0.05$ ) by pairwise comparison of the  $\chi^2$ .



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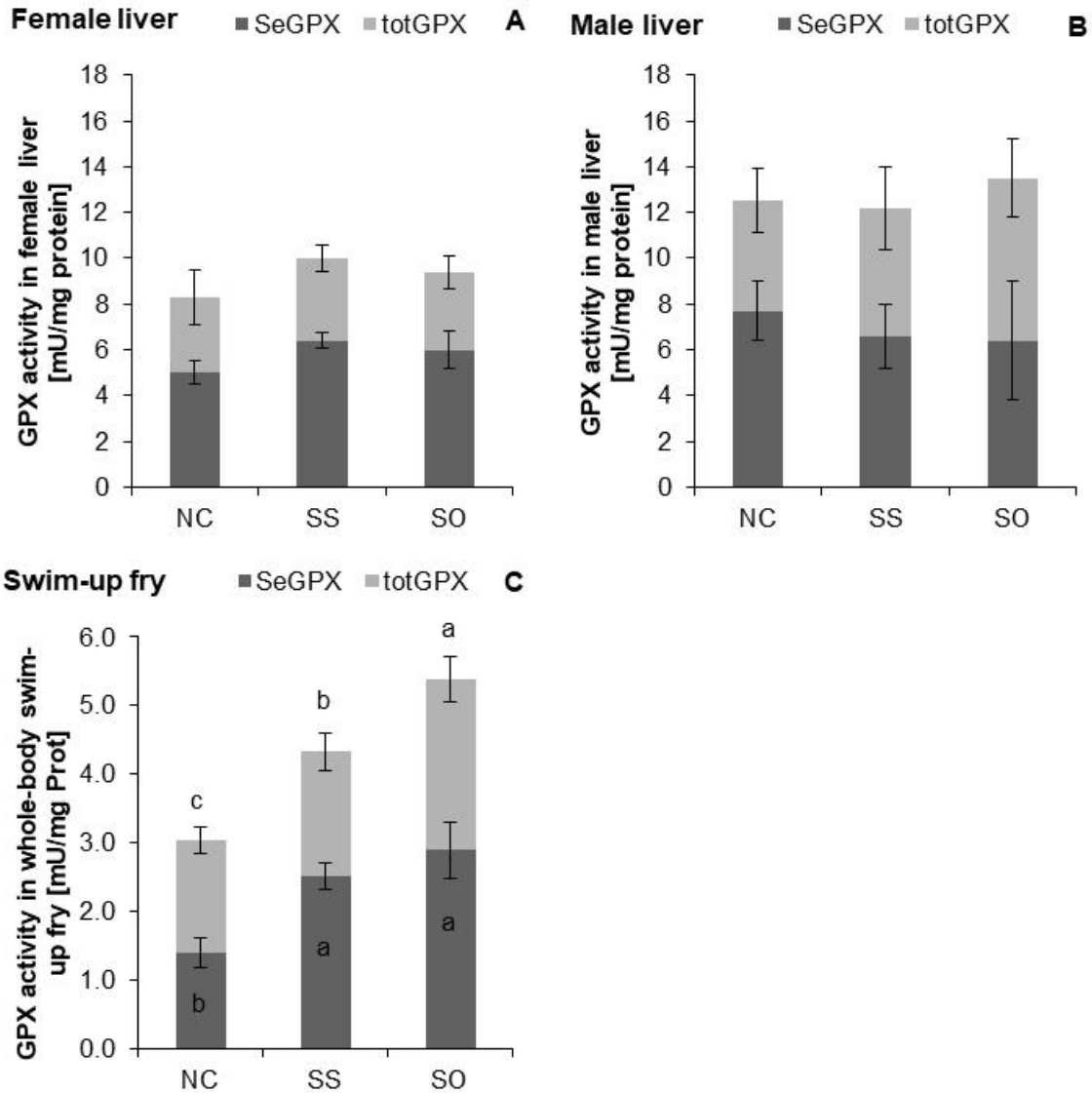
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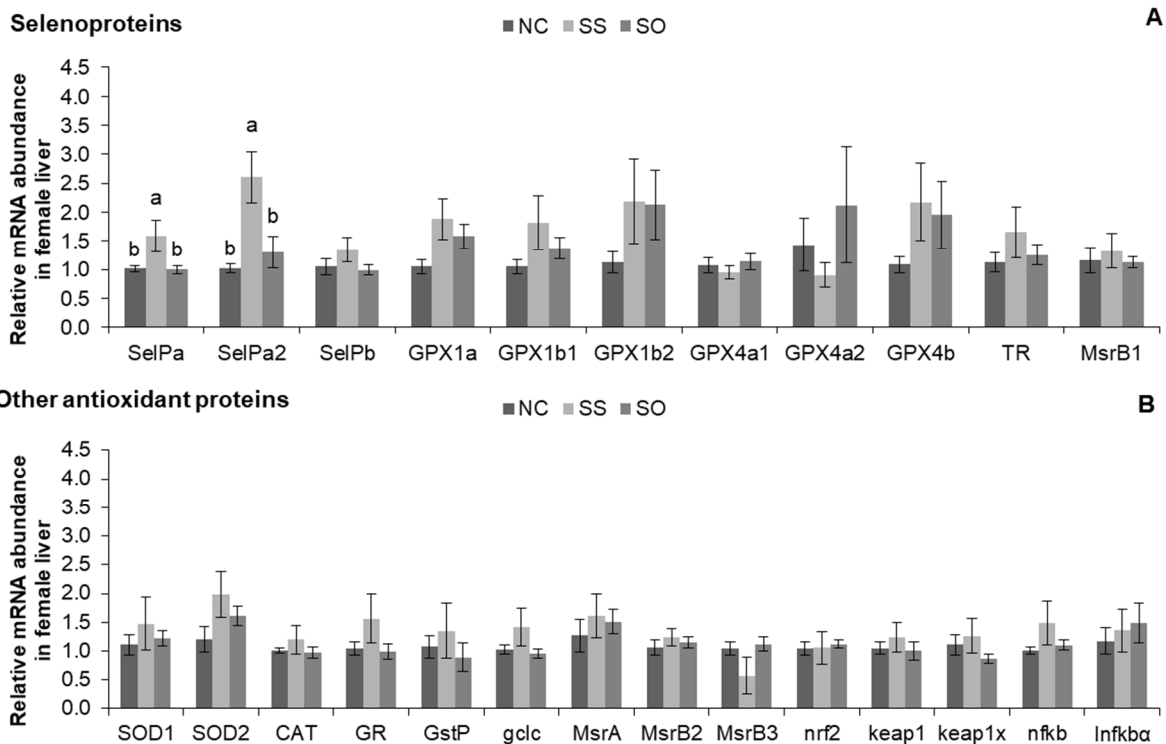
**Fig. 2.** Total Se content in female muscle (A), female liver (B) and in male milt (C) as well as in oocytes (D) and whole-body swim-up fry (E) from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of  $8 \pm 1^\circ\text{C}$ . Given as means  $\pm$  SEM ( $n = 8$ , except Se2 milt:  $n = 4$  and Se3 milt:  $n = 6$ ). Means not sharing a common superscript letter are significantly different ( $P < 0.05$ ) according to one-way ANOVA followed by Newman-Keuls post-hoc test.



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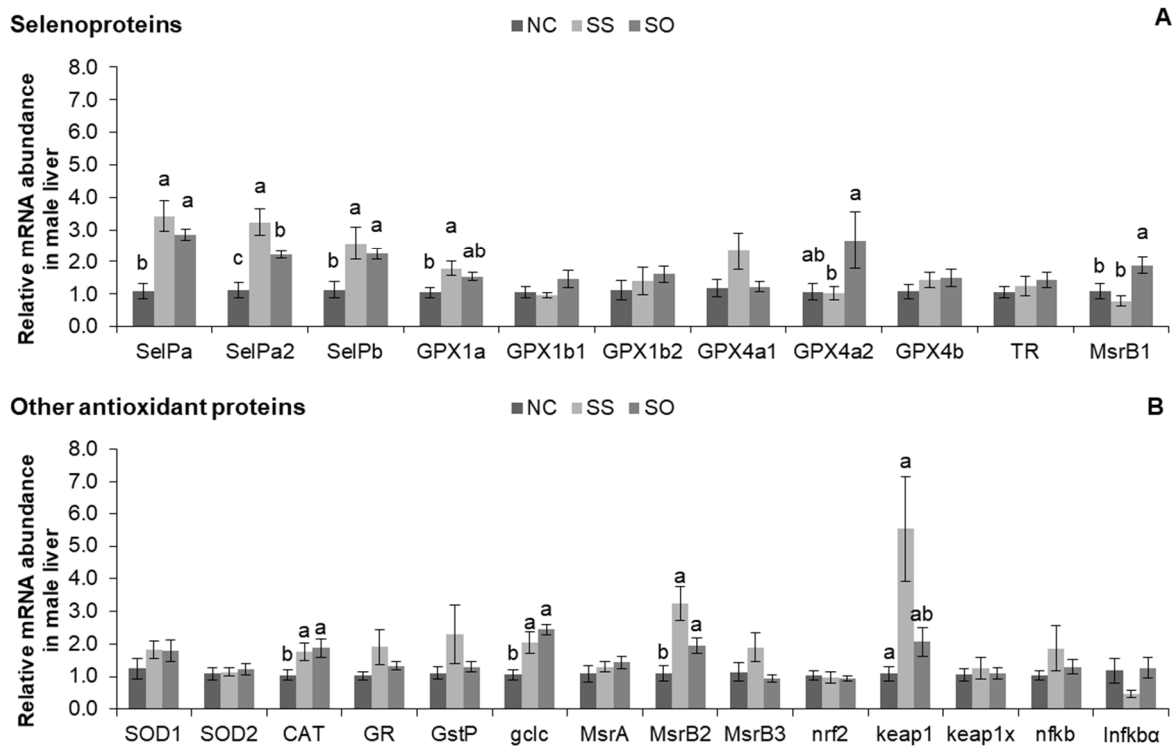
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**Fig. 3.** Total and Se-GPX activity in female liver (A), male liver (B) and whole-body swim-up fry (C) from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of  $8 \pm 1^\circ\text{C}$ . Bars represent means  $\pm$  SEM ( $n = 8$ , except for male liver:  $n = 5$ ). Means not sharing the same superscript letter are significantly different ( $P < 0.05$ ) according to one-way ANOVA followed by Newman-Keuls post-hoc test.



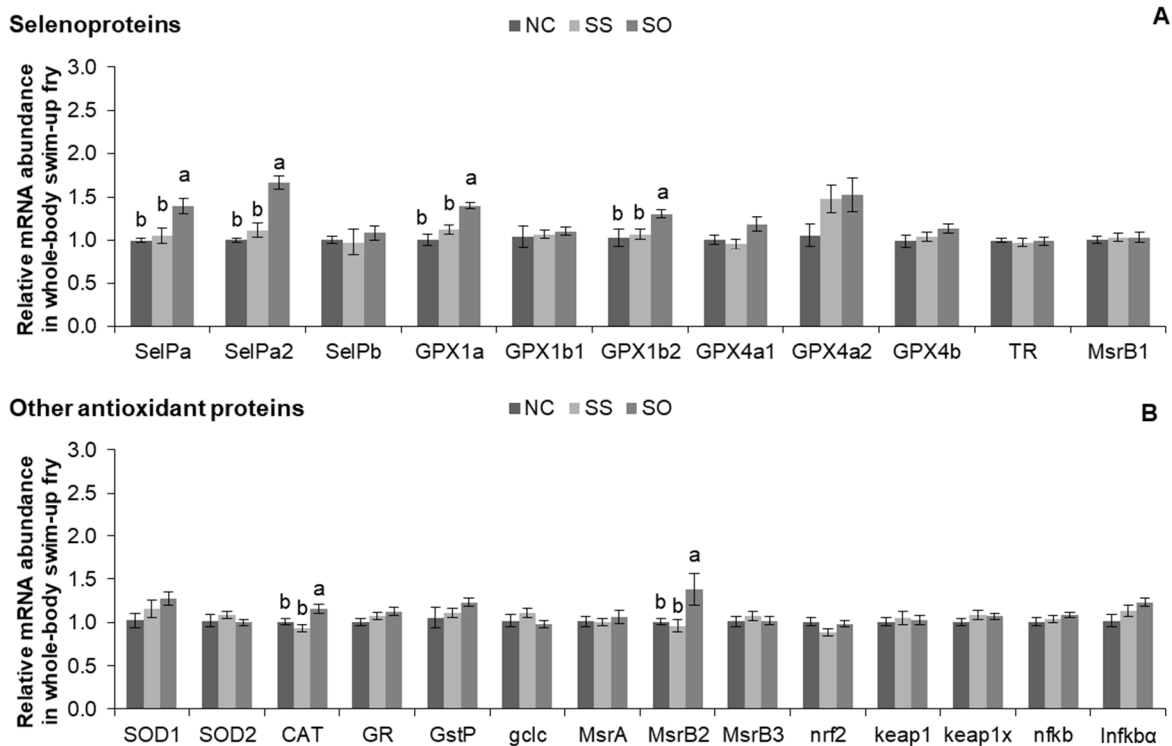
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 818 **Fig. 4.** Relative mRNA abundance of selenoproteins (A) and other proteins involved in  
 819 antioxidant metabolism (B) in female liver from rainbow trout broodstock fed different Se  
 820 sources over 6 months at a constant water temperature of  $8 \pm 1^\circ\text{C}$ . Data are normalized to  
 821  $\beta$ -actin and expressed as fold-changes of mRNA abundance compared with the control  
 822 group Se1. Bars represent means  $\pm$  SEM ( $n = 8$ ). Means not sharing a common superscript  
 823 letter are significantly different ( $P < 0.05$ ) according to one-way ANOVA followed by  
 824 Newman-Keuls test.

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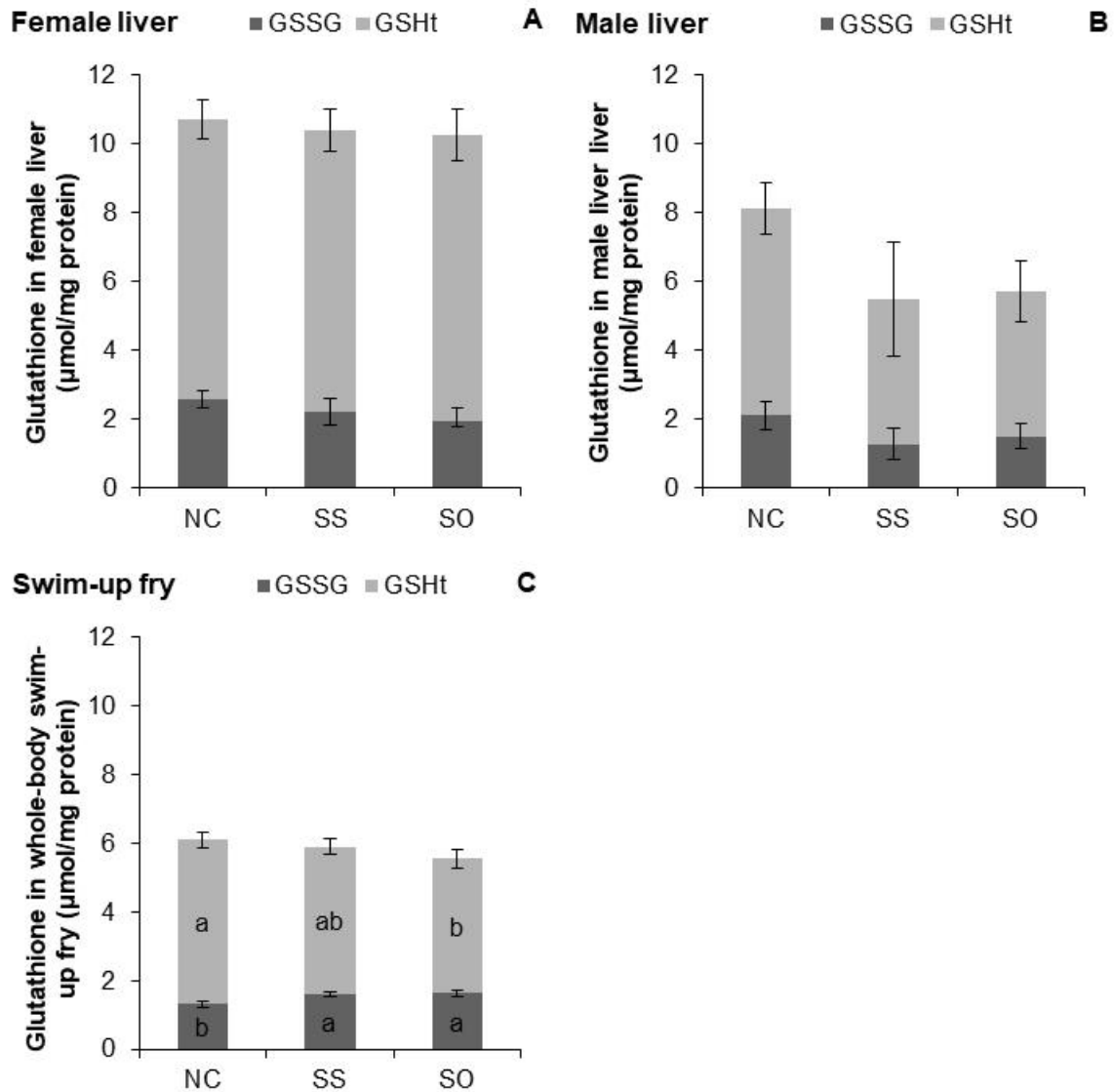
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**Fig. 5.** Relative mRNA abundance of selenoproteins (A) and other proteins involved in antioxidant metabolism (B) in male liver from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of  $8 \pm 1^\circ\text{C}$ . Data are normalized to  $\beta$ -actin and expressed as fold-changes of mRNA abundance compared with the control group Se1. Bars represent means  $\pm$  SEM ( $n = 5$ ). Means not sharing a common superscript letter are significantly different ( $P < 0.05$ ) according to one-way ANOVA followed by Newman-Keuls test.



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 836 **Fig. 6.** Relative mRNA abundance of selenoproteins (A) and other proteins involved in  
 837 antioxidant metabolism (B) in whole-body swim-up fry stage from rainbow trout broodstock  
 838 fed different Se sources over 6 months at a constant water temperature of  $8 \pm 1^\circ\text{C}$ . Data are  
 839 normalized to  $\beta$ -actin and expressed as fold-changes of mRNA abundance compared with  
 840 the control group Se1. Bars represent means  $\pm$  SEM ( $n = 8$ ). Means not sharing a common  
 841 superscript letter are significantly different ( $P < 0.05$ ) according to one-way ANOVA followed  
 842 by Newman-Keuls test.  
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846 **Fig. 7.** Glutathione contents in female liver (A), male liver (B), and whole-body swim-up fry  
 847 (C) from rainbow trout broodstock fed different Se sources over 6 months at a constant  
 848 water temperature of  $8 \pm 1^\circ\text{C}$ . Bars represent means  $\pm$  SEM ( $n = 8$ , except for male liver:  $n =$   
 849  $5$ ). Difference between total (GSht) and oxidized glutathione (GSSG) represents reduced  
 850 glutathione (GSH). Means not sharing a common superscript letter are significantly different  
 851 ( $P < 0.05$ ) according to one-way ANOVA followed by Newman-Keuls post-hoc test.  
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