

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

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4 Pauline Wischhusen^{a,*}, Maroussia Parailoux^a, Pierre-André Geraert^b, Mickael Briens^b, Maïté
5 Bueno^c, Sandra Mounicou^c, Brice Bouyssiere^c, Antony Jesu Prabhu^d, Sadasivam J.
6 Kaushik^{a,1}, Benoit Fauconneau^a, Stéphanie Fontagné-Dicharry^a,

7

8 Affiliations

9 ^aINRA, Univ Pau & Pays Adour, E2S UPPA, UMR 1419, Nutrition, Métabolisme,
10 Aquaculture, Saint Pée sur Nivelle, F-64310, France

11 ^bADISSEO, 10 Place du Général de Gaulle, 92160 Antony, France

12 ^cCNRS / Univ Pau & Pays Adour / E2S UPPA, Institut des Sciences Analytiques et de
13 Physicochimie pour l'Environnement et les Matériaux, UMR5254, 64000, Pau, France

14 ^dIMR, NO-5817 Bergen, Norway

15 ¹Present address: European Research Area (ERA) Chair, EcoAqua, Universidad de Las
16 Palmas de Gran Canaria, Taliarte, 35214 Telde, Las Palmas, Canary Islands, Spain

17

18 *Corresponding author Tel.: +33 5 59 51 59 51

19 E-mail address: pauline.wischhusen@inra.fr

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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 α -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

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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 ± 1 °C
105 with 25 females (initial mean weight: 1.1 ± 0.2 kg, second reproduction season) and 15
106 males (0.9 ± 0.3 kg) per tank (stocking density: 40 kg/m³). 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 ± 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 H₂O₂ (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 H₂O₂ in a solution of 67 mM phosphate buffer, 1 mM
187 EDTA and 20 mM H₂O₂ (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)₁₅ 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 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 β -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 g
215 homogenized liver tissues and 1 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 χ^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 ± 2 to 15 ± 1 ng Se per individual)
285 and SO (29 ± 3 to 23 ± 1 ng Se per individual) groups whereas no significant change in Se
286 content was noticed for NC (11 ± 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 α -
340 tocopherol concentration per individual in the offspring. The concentrations of γ - and δ -
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 δ -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 α -
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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716 concentration and expression by different sources of selenium in broiler breeders and
717 their offspring. *Poult. Sci.* 92, 2375–2380.
- 718 Zhang, L., Wang, Y.X., Zhou, Y., Zheng, L., Zhan, X.A., Pu, Q.H., 2014. Different sources of
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 ¹	74	74	74
Crystalline amino acids and attractant mixture ²	3.14	3.14	3.14
Soybean lecithin ³	2	2	2
Fish oil ³	8	8	8
Vegetable oils ⁴	8	8	8
Carophyll® pink ⁵	0.04	0.04	0.04
Vitamin and mineral mixture without Se ⁶	4.82	4.82	4.82
Sodium selenite (µg/g diet) ⁷	-	0.7	-
Selisseo® (µg/g diet) ⁷	-	-	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 ⁸	35.1	33.6	35.3
16:1	3.8	3.5	3.7
18:1	28.0	28.8	27.5
Total monoenes ⁸	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 ¹Plant 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 ²Crystalline 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 ³Soybean lecithin from Louis François and fish oil from Sopropêche.

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

732 ⁵Contained 10% astaxanthin (DSM).

733 ⁶Vitamin and mineral mixture without Se (per kg diet): retinol acetate, 55,000 IU;
734 cholecalciferol, 2,500 IU; DL- α -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; CaHPO₄·2H₂O, 33 g; CaCO₃, 2.15
738 g; Mg(OH)₂, 1.24 g; KCl, 0.9 g; NaCl, 0.4 g; FeSO₄·7H₂O, 0.2 g; ZnSO₄·7H₂O, 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 α -cellulose.

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

743 ⁸Total 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	cctggagtagggccacca	82
SeIPa2	MH085054.1	accttgctgagccagaaact	cagacgaccacacctgtcat	129
SeIPb	HF969250.2	gtgtcgttcctcatcgtgaa	ggaacgtcagtcctccacat	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	tgatgtcacacaggtgcgta	gtgggctcagtggtgtgag	195
GR	HF969248.1	ctaagcgcagcgtcatagtg	acaccctgtctgacgacat	108
Gclc	GSONMT00065033001	caaccaactggcagacaatg	ccttgacaaggggatgaga	189
GST π	BX302932.3	tcgctgactggacgaaagga	cgaaggtcctcaacgccatc	196
Nrf2	CA360709.1	tgagctgcagcaatgtctga	ggtgggcaatgggtagaagc	124
Keap1	GSONMT00034445001	gctacgtgatgtctgccct	ggtacctcatagcggccagt	116
Keap1X	GSONMT00019673001	actgggaggttatgacggga	cctccgtcctctcatgcttc	179
NF- κ B	BX880658.3	cagcgtcctaccaggctaaagagat	gctgttcgatccatccgactat	181
I κ B α	BT074199.1	agagacagactgcgtccac	cggcctcagtagcctctct	72
β -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 π , glutathione S-transferase π ; Nrf2, nuclear factor erythroid-2 related factor 2; Keap1,
750 Kelch-like ECH-associated protein 1; NF- κ B, nuclear factor kappa-light chain-enhancer of
751 activated B cells; I κ B α , NF- κ B inhibitor α .
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°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 ¹ (% , n=9-11)	0.34 \pm 0.08	0.28 \pm 0.14	0.29 \pm 0.12
HSI ¹ (% , 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 ¹ (% , n=10-21)	0.27 \pm 0.05 ^{ab}	0.17 \pm 0.04 ^b	0.32 \pm 0.05 ^a
HSI ¹ (% , 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 ¹ (% , 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 ^{ab}	76 \pm 6 ^b	92 \pm 1 ^a
Survival at swim-up fry stage (% , n=12-21)	87 \pm 2 ^{ab}	75 \pm 6 ^b	91 \pm 1 ^a
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 ¹DGI, 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 ^c	118 \pm 18 ^c	89 \pm 8	0.55 \pm 0.06	0.42 \pm 0.04 ^a	1.35 \pm 0.25 ^b	97 \pm 3
SS	321 \pm 14 ^b	220 \pm 24 ^b	102 \pm 14	0.69 \pm 0.06	0.32 \pm 0.03 ^{ab}	2.24 \pm 0.38 ^{ab}	100 \pm 3
SO	521 \pm 19 ^a	373 \pm 5 ^a	116 \pm 19	0.72 \pm 0.02	0.22 \pm 0.03 ^b	3.40 \pm 0.56 ^a	94 \pm 1
<i>Swim-up fry</i>							
NC	197 \pm 17 ^b	47 \pm 9 ^b	13 \pm 2	0.24 \pm 0.06	0.07 \pm 0.01	3.68 \pm 0.77 ^b	31 \pm 6
SS	276 \pm 11 ^a	40 \pm 5 ^b	13 \pm 1	0.15 \pm 0.03	0.05 \pm 0.01	2.95 \pm 0.03 ^b	19 \pm 4
SO	312 \pm 10 ^a	84 \pm 6 ^a	12 \pm 2	0.27 \pm 0.01	0.04 \pm 0.00	6.89 \pm 0.78 ^a	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 ¹	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 ¹	409 ± 32	373 ± 45	391 ± 50	502 ± 60	574 ± 52	424 ± 49	11 ± 1	11 ± 1	11 ± 1
GR ²	8 ± 1	9 ± 1	9 ± 1	12 ± 2	7 ± 1	11 ± 1	8 ± 1	8 ± 1	8 ± 1
GST ²	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 ¹Expressed in U/mg protein

778 ²Expressed 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 $8 \pm 1^\circ\text{C}$.

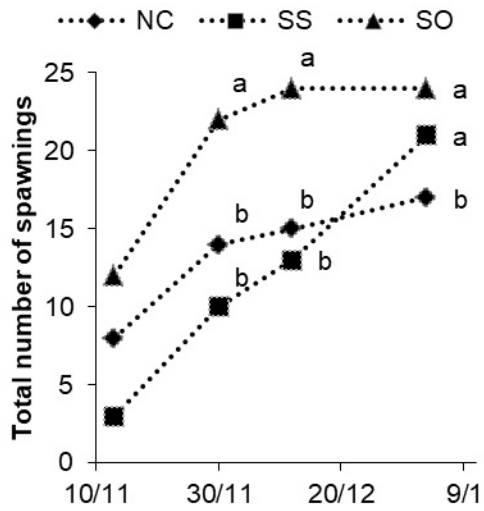
	NC	SS	SO
GSH:GSSG	3.7 ± 0.3^a	2.7 ± 0.2^b	2.4 ± 0.1^b
8-Isoprostanes (pg/g)	44 ± 2^a	35 ± 3^b	50 ± 5^a
Protein carbonyls (nmol/mg protein)	19 ± 1	19 ± 1	16 ± 1
Vitamin C (mg/kg)	29 ± 1^b	30 ± 2^b	37 ± 2^a

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°C .

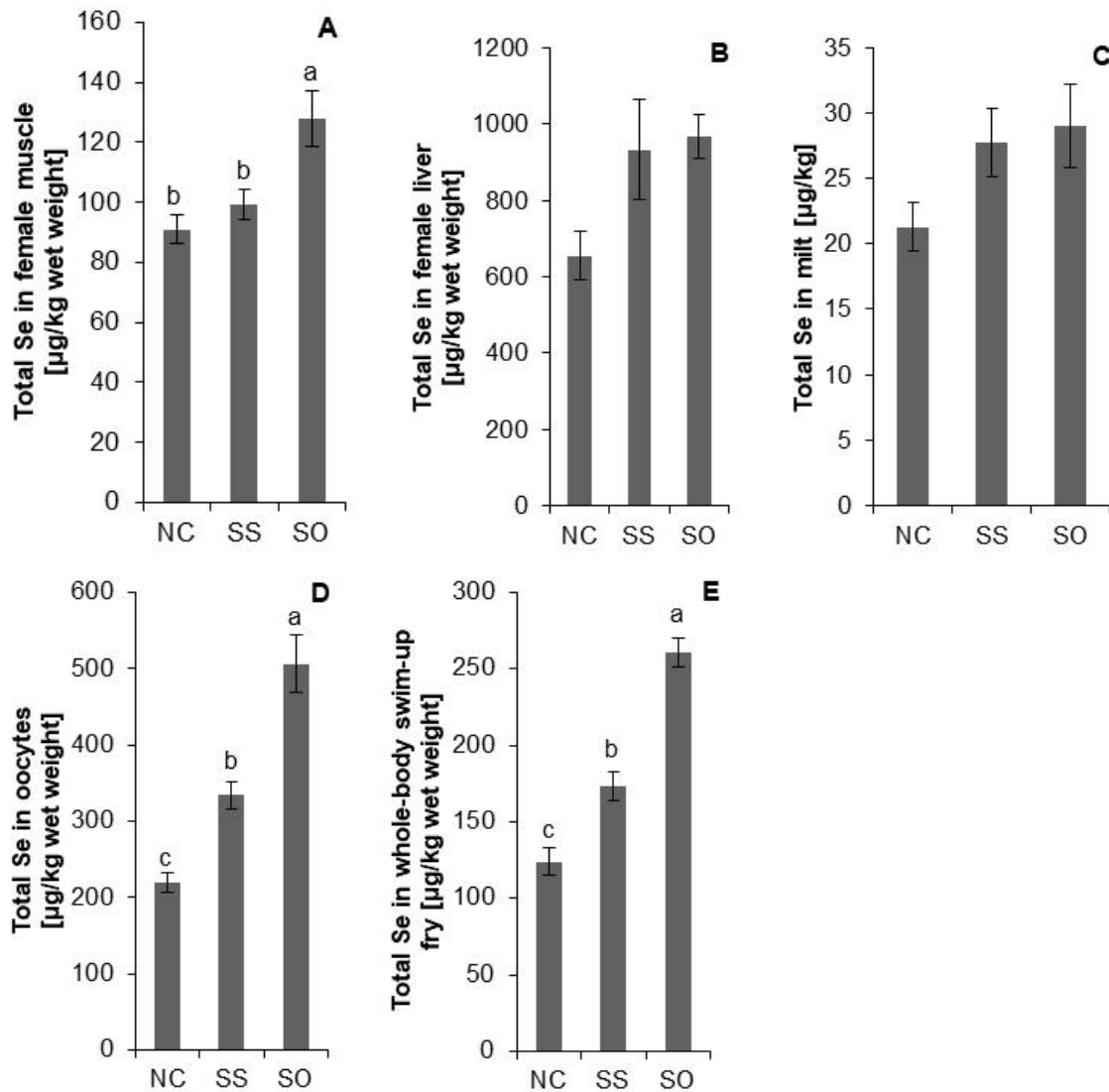
	<u>Dietary group</u>			<u>Stage</u>		<u>p-value</u>		
	NC	SS	SO	oocyte	swim-up fry	Diet	Stage	DxS
α -tocopherol	1815 ± 146^b	2056 ± 176^{ab}	2472 ± 168^a	2157 ± 154	2071 ± 132	0.043	0.773	0.794
γ -tocopherol	254 ± 23	249 ± 26	254 ± 15	263 ± 17	241 ± 18	0.348	0.426	0.400
δ -tocopherol	5.15 ± 0.68	5.67 ± 0.69	4.68 ± 0.66	6.31 ± 0.36^a	4.02 ± 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 χ^2 .



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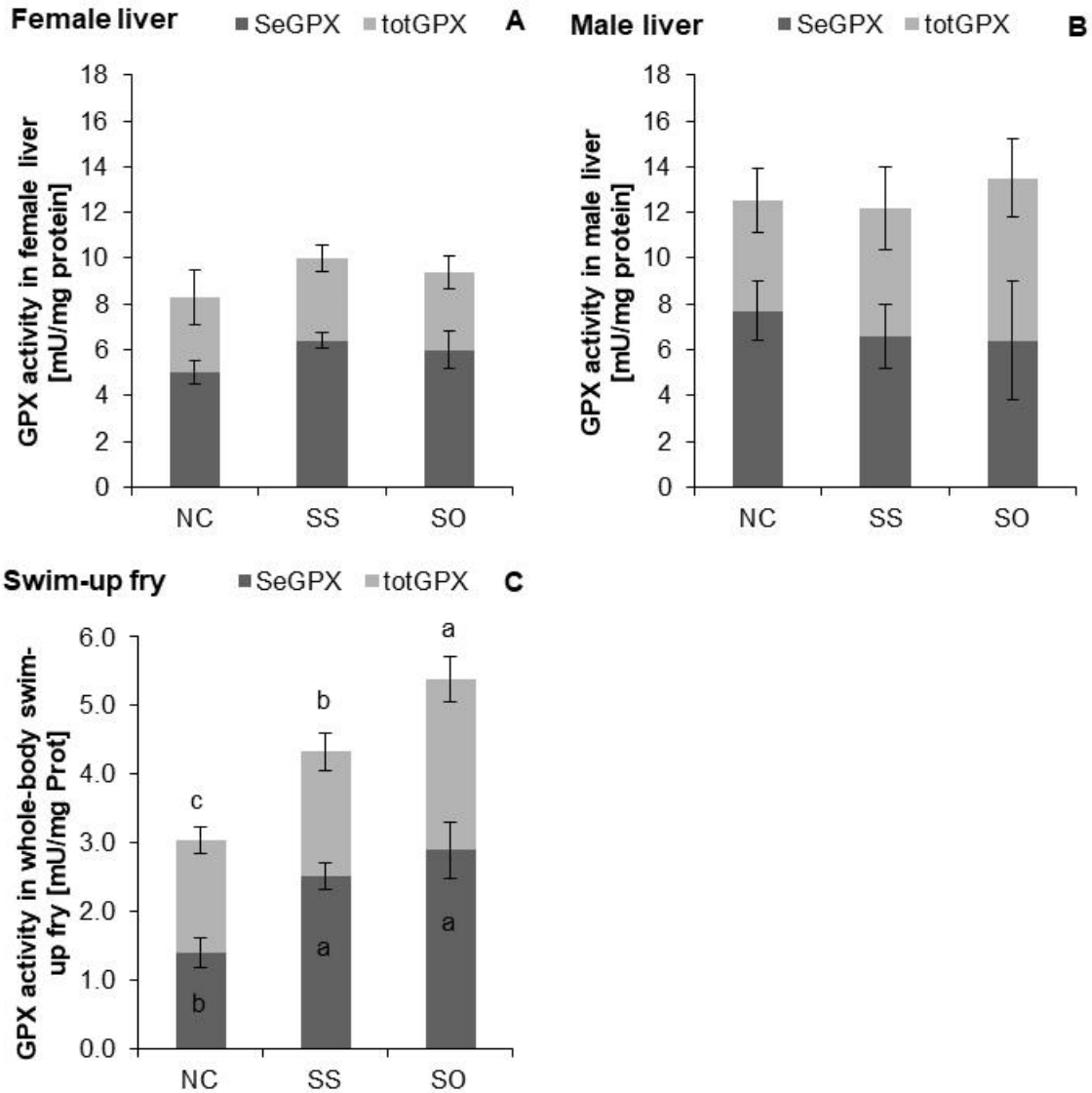
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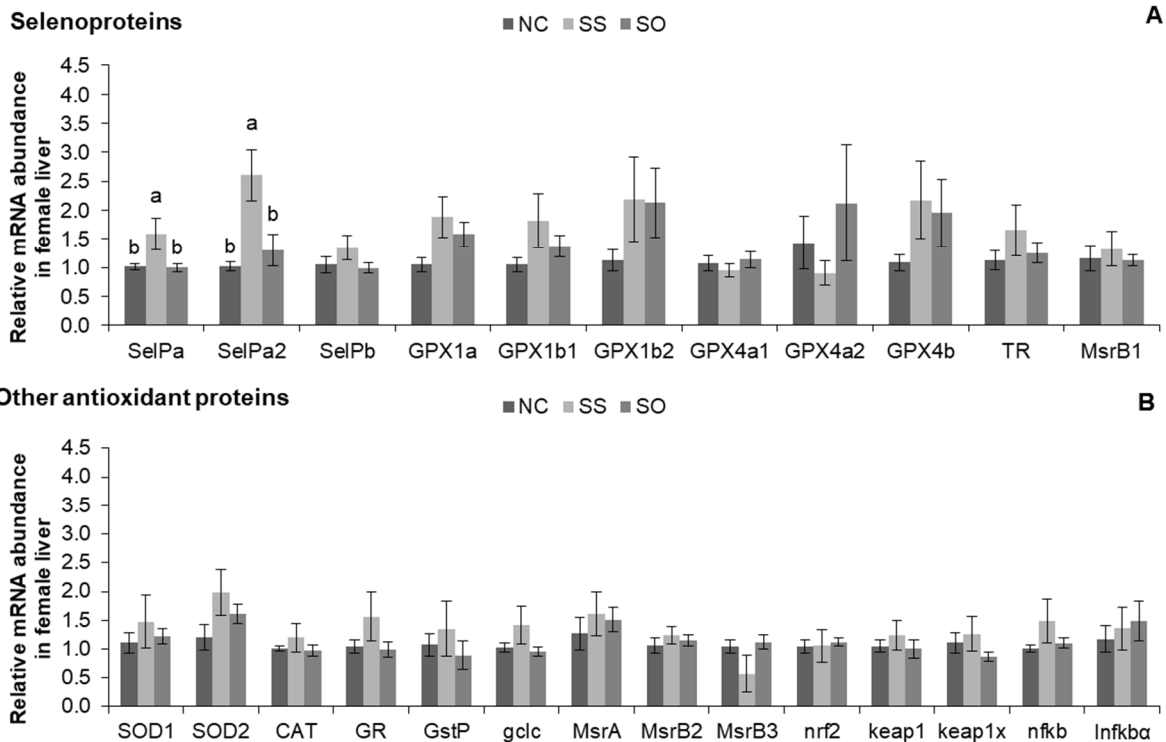
Fig. 2. Total Se content in female muscle (A), female liver (B) and in male milt (C) as well as in oocytes (D) and whole-body swim-up fry (E) from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of $8 \pm 1^\circ\text{C}$. Given as means \pm SEM ($n = 8$, 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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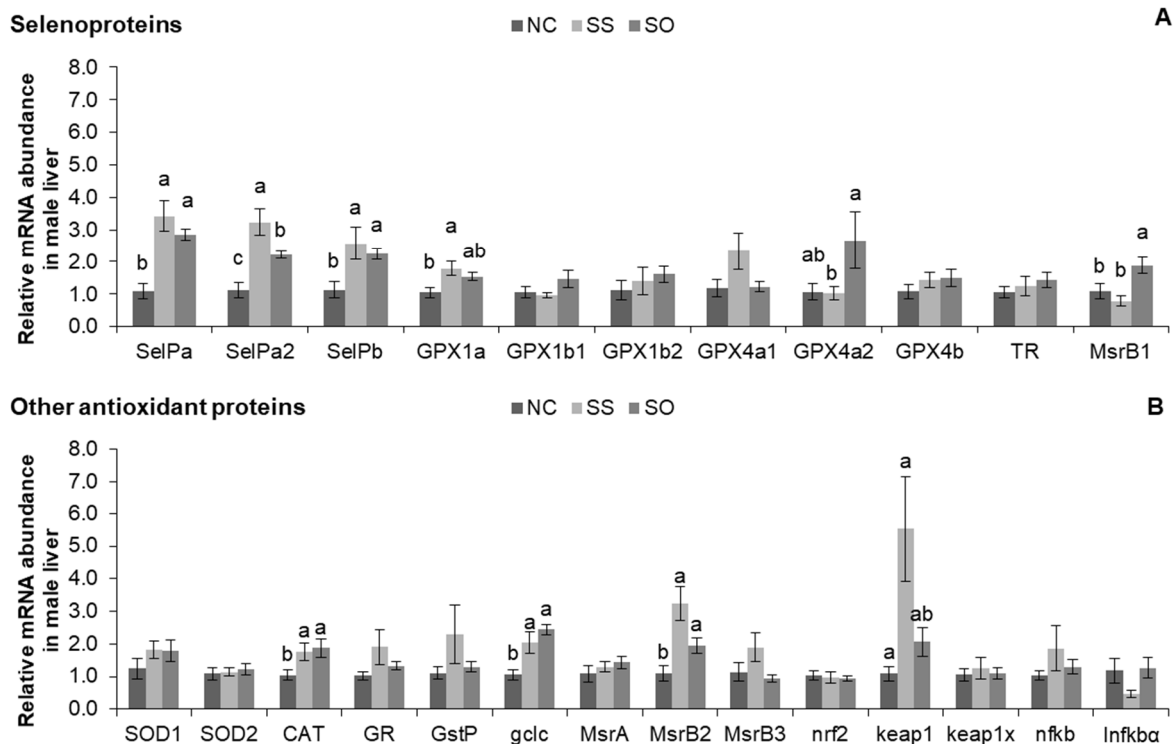
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Fig. 3. Total and Se-GPX activity in female liver (A), male liver (B) and whole-body swim-up fry (C) from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of $8 \pm 1^\circ\text{C}$. Bars represent means \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.



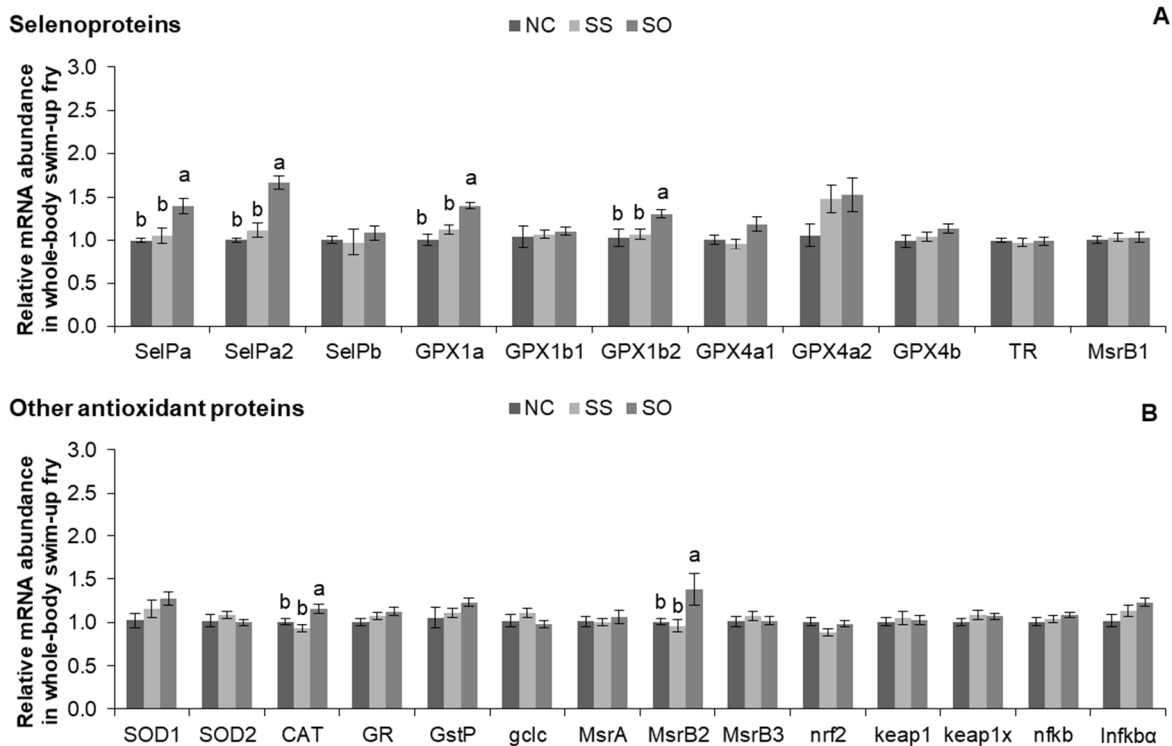
817
 818 **Fig. 4.** Relative mRNA abundance of selenoproteins (A) and other proteins involved in
 819 antioxidant metabolism (B) in female liver from rainbow trout broodstock fed different Se
 820 sources over 6 months at a constant water temperature of $8 \pm 1^\circ\text{C}$. Data are normalized to
 821 β -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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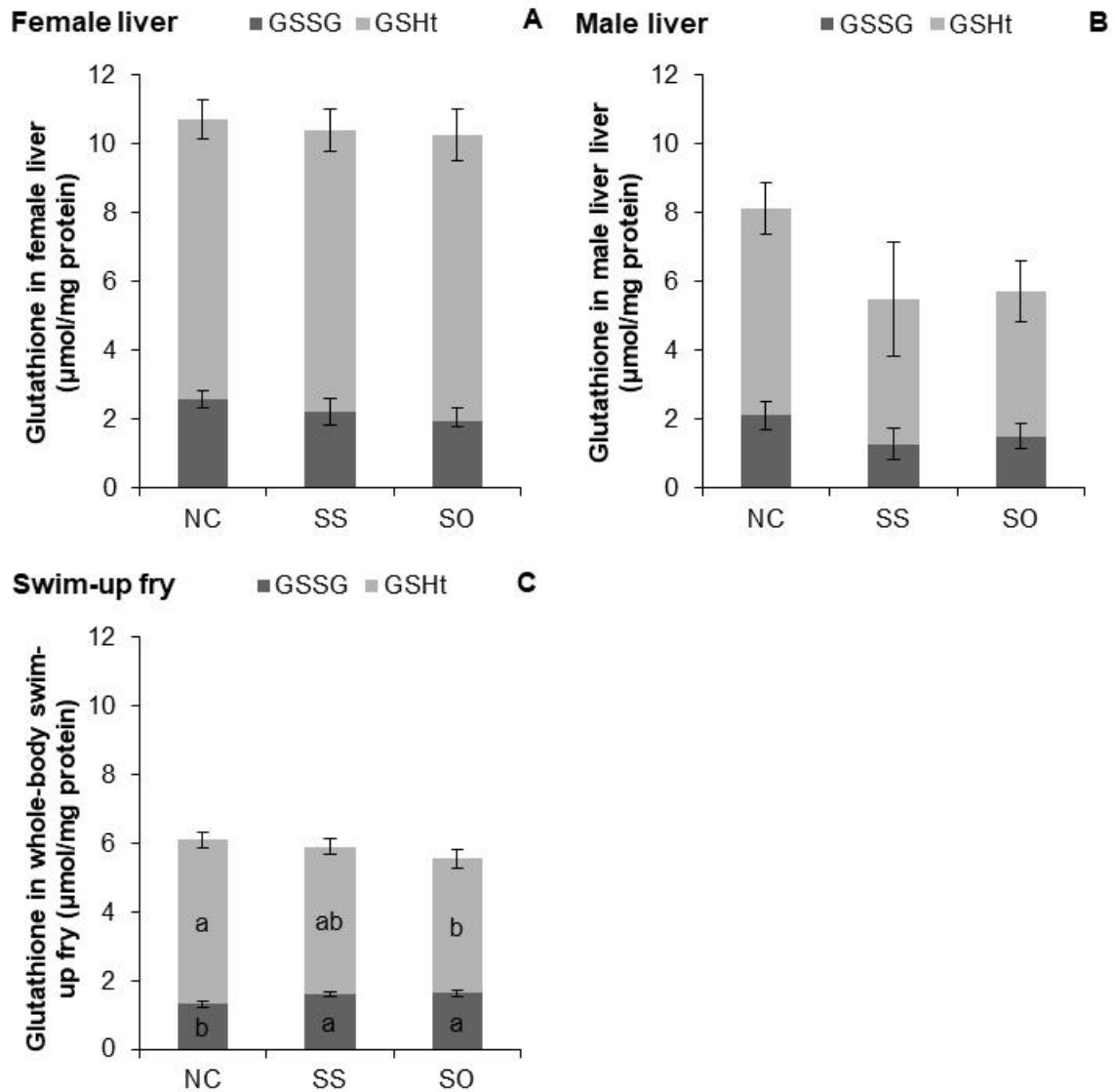


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Fig. 5. Relative mRNA abundance of selenoproteins (A) and other proteins involved in antioxidant metabolism (B) in male liver from rainbow trout broodstock fed different Se sources over 6 months at a constant water temperature of $8 \pm 1^\circ\text{C}$. Data are normalized to β -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.



835
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 β -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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845

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.
 852