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1 **Dietary antioxidants, food deprivation and growth affect differently oxidative**
2 **status of blood and brain in juvenile European seabass (*Dicentrarchus labrax*)**

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22 **Running title:** Early life and oxidative stress

23 **ABSTRACT**

24 Compensatory growth may increase molecular oxidative damage, which may be
25 mitigated through the intake of dietary antioxidants. However, dietary antioxidants may
26 also reduce concentration of antioxidant enzymes, which have a key role in regulating
27 the oxidative status. Here we investigated whether feeding on a diet rich in antioxidants
28 (vitamin E) enables juvenile European seabass (*Dicentrarchus labrax*) to catch up after
29 a period of food deprivation with negligible effects on the oxidative stress to blood and
30 brain as compared to fish feeding on a normal diet (i.e., not enriched in antioxidants).
31 The results show that a higher intake in antioxidants favoured compensatory growth, but
32 this came at a cost in terms of increased oxidative damage. Increased intake of
33 antioxidants also resulted in changes in the activity concentration of enzymatic
34 antioxidant defences and increased protein oxidative damage in both brain and blood. In
35 addition, food deprivation caused increased protein oxidative damage in brain. Our
36 findings show that the beneficial effects of dietary antioxidants on growth may be offset
37 by hidden detrimental effects and that different early life events affect different
38 components of oxidative status of a given tissue.

39

40 *Keywords:* Antioxidants, Aquaculture, Early life, Fish, Food restriction, Oxidative
41 stress

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44 **1. Introduction**

45 Positive selection of individuals capable of reaching an adult size quickly should be
46 expected to sift out slowly growing individuals. Achieving an adult size as soon as
47 possible should carry potential benefits to the individual, such as earlier time to sexual
48 maturity or reduced predation risk (Dmitriew, 2011). Conversely to this prediction,
49 animals do not grow at the maximum rate and much variation in growth strategies
50 persists in both wild and laboratory animals, even when trophic resources are abundant
51 (Blanckenhorn, 2000). The reason for this might lie with the costs of growing fast
52 (Metcalf and Monaghan, 2001). For example, rapid growth can increase metabolic
53 demands (Crisuolo et al., 2008; Careau et al., 2013) and daily energy expenditure
54 (Careau et al., 2013), or reduce body performance and lifespan (Lee et al., 2016). An
55 additional mechanism through which growth may relate to self-maintenance is that
56 increased cellular activity due to fast growth increases the production of reactive
57 species, such as free radicals (Costantini, 2014; Smith et al., 2016). These unstable
58 molecules, if not counteracted by antioxidants, oxidise proteins, lipids and nucleic acids,
59 causing damage, and hence a state of oxidative stress (Halliwell and Gutteridge, 2015).
60 Recent correlative, experimental and meta-analytical work has suggested that oxidative
61 stress might play a key role as a constraint on, and cost of, growth (Alonso-Alvarez et
62 al., 2007; Nussey et al., 2009; Smith et al., 2016).

63 Oxidative costs of a rapid growth are particularly relevant when individuals
64 accelerate growth to achieve the body size that is supposed to be normal for their life
65 stage (known also as catch-up growth; Metcalf and Monaghan, 2001). For example,
66 abrupt reductions in food availability - common in wild populations - might lead young
67 to slow down growth. When food availability becomes adequate again to meet the

68 nutritional requirements of a growing individual, juveniles will compensate by
69 accelerating their growth. Although there are benefits in doing so, an acceleration of
70 growth may also carry costs, such as a reduction in motor skills, sexual ornamentation,
71 cognitive abilities or longevity (Metcalf and Monaghan, 2001; Lee et al., 2016), but
72 also in increased molecular oxidative damage (Smith et al., 2016).

73 Organisms may mitigate oxidative stress through an increased intake of
74 antioxidants from diet. Animals normally ingest a cocktail of antioxidants and distribute
75 them differently across tissues, depending on the chemical properties (lipophilic vs.
76 hydrophilic) of the antioxidant and the specific requirements of a certain tissue (Surai,
77 2002; Halliwell and Gutteridge, 2015). Dietary antioxidants may be particularly
78 important for growing individuals as the endogenous antioxidant machinery takes time
79 to become fully mature (Surai, 2002; Costantini, 2014). In many vertebrate species,
80 there is a greater reliance upon non-enzymatic antioxidants at earlier stages of
81 development, such as those acquired from diet or from the mother (Surai, 2002;
82 Costantini, 2014). It would therefore be expected that if an individual catches up while
83 feeding on a diet rich in antioxidants, it might mitigate the oxidative costs of a fast
84 growth. This simple prediction, however, does not consider that increases in the intake
85 of a given antioxidant may also result in no net reduction in oxidative damage because
86 any potential beneficial effects might be offset by compensatory reductions in other
87 antioxidants. For example, long-term intake of a dietary antioxidant (vitamin C) in
88 laboratory mice reduced expression of several genes linked to free radical scavenging,
89 with no net effect on various oxidative damage metrics (Selman et al., 2006). A recent
90 meta-analysis also showed that the effects of antioxidant supplementation on growth are

91 highly variable (from positive to negative), implying that effects of dietary antioxidants
92 on oxidative status might be difficult to predict (Smith et al., 2016).

93 Another aspect that has received little attention is the impact that accelerated
94 growth has on brain oxidative status and protein oxidative damage (Smith et al., 2016).
95 This is surprising given that acceleration of growth may reduce cognitive abilities
96 (Metcalf and Monaghan, 2001), brain is a tissue particularly sensitive to oxidative
97 stress (Barja, 2004) and protein turnover is altered during fast growth (Samuels and
98 Baracos, 1995).

99 Compensatory growth is particularly common in fish at almost every stage of
100 their life cycles because of their indeterminate growth patterns (Ali et al., 2003).
101 Despite the expansion of the field of ecological oxidative stress, relatively little is
102 known about the role of oxidative stress in mediating key life-history stages of fish
103 (Birnie-Gauvin et al., 2017), such as those fish go through during growth and
104 development.

105 In this study, we tested experimentally whether feeding on a diet rich in
106 antioxidants enables young European seabass (*Dicentrarchus labrax*) to catch up after a
107 period of food deprivation with negligible effects on the oxidative stress as compared to
108 fish feeding on a normal diet (i.e., not enriched in antioxidants). To this end, we have
109 measured metrics of oxidative damage and of antioxidant enzyme activity (i.e., proxy of
110 enzyme concentration) in both blood and brain in juvenile fish that underwent a food
111 deprivation period and then a refeeding protocol either with or without an enriched diet.

112

113 **2. Materials and methods**

114 *2.1. Ethical note*

115 All experiments complied with the Guidelines of the European Union Council and were
116 approved by the Ethical Committee of the Tuscia University for the use of live animals
117 (D.R. n. 677/16 and D.R. 644/17).

118 At the end of the food deprivation period, the loss of body mass in fish that were
119 food-deprived was 11.4 and 12.6 % for the two experimental groups (mean \pm se: prior
120 food deprivation, 59.7 ± 5.1 g and 59.1 ± 5.1 g; end of food deprivation, 52.8 ± 5.2 g
121 and 51.6 ± 5.2 g), respectively. Hence, it was below the threshold of 15 %
122 recommended by the UK Home Office and the Canadian Council for Animal Care. The
123 mortality rates calculated using sample sizes at the beginning of Phase 1 were similar
124 among groups at the end of the experiment: 12.5 % (number of fish dead were 3), 25.0
125 % (number of fish dead were 7), 14.3 % (number of fish dead were 4) and 14.3 %
126 (number of fish dead were 4) for no food deprivation/standard diet, no food
127 deprivation/enriched diet, food deprivation/standard diet, and food deprivation/enriched
128 diet, respectively.

129

130 2.2. *Animals and husbandry*

131 Juvenile European seabass ($n = 112$, one year of age) were obtained from a commercial
132 hatchery (Cooperativa Orbetello Pesca Lagunare, Santa Liberata, Orbetello, Italy)
133 located on the coast at about 40 km from the facility where the study took place. The
134 fish were transported to the facility and randomly allocated into four fiberglass tanks
135 (80 x 80 x 403 cm, all equipped with aeration system) in groups of similar size (26, 29,
136 29 and 28 individuals). Before being released into the tanks, each fish was massed and
137 measured (mean \pm SD, body mass: 64.5 ± 22.7 g; body length 18.9 ± 2.0 cm; body
138 width 4.2 ± 0.5 cm, see below for method details). In each tank, fish were maintained in

139 a volume of 1,290 liters on an open circuit system with flow of 30 liters/minute
140 (replicating the conditions of the supplier hatchery) on natural light dark cycle and
141 illumination, as ample windows were present in the building. Being an open circuit
142 meant that each tank was subject to natural seasonal variations of chemical/physical
143 parameters: pH ranged between 8.0 and 8.2, temperature varied from 15°C in January to
144 23°C in August, while salinity ranged between 35 and 38 PSU; ammonia and nitrite
145 were below detectable levels, while nitrate was about 5 mg/L. The European seabass is
146 an euryhaline coastal species known to tolerate (at both juvenile and adult stage) a large
147 range of chemical-physical water parameters, including salinity and temperature (in the
148 order of 5-28°C) extremes (e.g. Dulger et al., 2012).

149 Tanks were cleaned daily to avoid accumulation of food remains. Fish were fed
150 by hand *ad libitum* once per day on the same commercial standard feed (company name
151 undisclosed) used by the supplier for 12 acclimation days. In order to make sure the
152 ration was *ad libitum*, we provided food until fish did not eat pellets anymore. The
153 residual pellets that deposited at the bottom of the tank were removed within around 60
154 minutes in order to minimise any potential confounding effects arising from very
155 bold/dominant individuals monopolising the access to the food. The feed composition
156 was as follows: crude proteins 48.0 %; crude fats and oils 22.0 %; crude fibre 3.0 %;
157 crude ash 6.0 %; vitamin E 150 mg/Kg.

158

159 *2.3. Experimental procedure and sampling*

160 Phase 1, food deprivation: after the acclimation period, the fish of two tanks started a
161 food deprivation protocol that lasted 81 days, while the control fish in the other two
162 tanks continued to be fed daily on the same commercial feed as previously (Fig. 1). All

163 fish were massed and measured on the day Phase 1 was started; the sample size was 108
164 because four fish died before the start of Phase 1.

165 Phase 2, re-feeding and enriched diet: as the food deprivation protocol stopped,
166 the pool of animals was re-fed as follows: two groups were fed daily on the standard
167 feed, and the other two groups were fed by hand *ad libitum* once per day on a feed
168 enriched with vitamin E (feed composition: crude proteins 48.0 %; crude fats and oils
169 20.0 %; crude fibre 2.0 %; crude ash 6.0 %; vitamin E 600 mg/Kg). In this way any
170 effect of the enriched diet on growth and oxidative status could be tested on both the
171 food restricted and the control fish (Fig. 1). This phase lasted until the final sampling,
172 which occurred 180 days later.

173 In order to quantify the growth, fish were caught with hand nets by two
174 operators, massed (Sartorius TE 612) and measured with a caliper for length and body
175 width (excluding dorsal and ventral fins) by a third operator once at the end of phase 1
176 and three times during phase 2 (Fig. 1). At the final sampling, the animals were bled
177 after a mild anesthesia and were straightaway euthanized with an overdose of 2-
178 phenoxyethanol (8 ml/l). Soon after euthanisation, the brain was collected from each
179 individual. Tubes containing blood samples were spun to separate plasma from red
180 blood cells. All samples were stored at -80°C and later transported in dry ice to the
181 laboratories of the University of Antwerp for the biochemical analyses.

182

183 2.4. Laboratory analyses

184 We randomly selected samples of blood and brain of 10 individuals per experimental
185 group. Whole brain was homogenised in cold PBS (supplemented with 20% (v/v) of
186 glycerol and with 0.2 mM of phenylmethylsulfonyl fluoride as an inhibitor of proteases)

187 using a pestle. Samples were then sonicated for 10 minutes and then centrifuged for 10
188 minutes at 15,000 rpm. The supernatant was taken and split into different tubes, which
189 were stored at -80°C for later analyses. Haemolysates were centrifuged to separate cell
190 membranes from the supernatant, which were used for the analyses straightaway. The d-
191 ROMs assay (reactive oxygen metabolites; Diacron International, Grosseto, Italy) was
192 used to measure plasma oxidative damage metabolites (e.g., organic hydroperoxides)
193 that are generated early in the oxidative cascade. Values were expressed as mM H₂O₂
194 equivalents. Protein carbonyls (marker of oxidative damage to proteins) were measured
195 in plasma and brain homogenates using the Protein Carbonyl Colorimetric assay
196 (Cayman Chemical Company, Ann Arbor, MI, USA). The assay is based on the
197 protocol of Levine et al. (1990). The concentration of protein carbonyls has been
198 expressed as nmoles/mg proteins. The Ransod assay (RANDOX Laboratories, Crumlin,
199 UK) was used to quantify the activity of the enzyme superoxide dismutase (SOD) in
200 both red blood cells and brain. The activity of SOD has been expressed as Units/mg
201 proteins. The Ransel assay (RANDOX Laboratories, Crumlin, UK) was used to
202 quantify the activity of the enzyme glutathione peroxidase (GPX) in both red blood cells
203 and brain. The activity of GPX has been expressed as Units/mg proteins. The assay is
204 based on the original method of Paglia and Valentine (1967). Protein concentration in
205 the haemolysate or homogenate was measured using the Bradford protein assay (Bio-
206 Rad Laboratories, Hercules, CA, USA).

207

208 *2.5. Statistical analyses*

209 Statistical analyses were performed using SPSS Version 23. General Linear Models
210 were used to test the effect of our experimental manipulation on growth. Three main

211 factors and their interactions were included in the model. The main factors were pre-
212 refeeding group (fed or food deprived), refeeding group (food enriched or not enriched
213 with antioxidants) and sampling day (three levels). As a response variable (body size
214 index), we used the first principal component (PC1, 95.8 % of the total variance)
215 extracted from a principal component analysis (PCA) on body mass, body length and
216 body width. The loadings (expressed as Pearson correlation coefficients) of these three
217 body measurements on the PC1 were as follows: 0.984 for body mass, 0.984 for body
218 length and 0.969 for body width. Analyses were repeated including body length or body
219 size (PC1 of a PCA on body mass and body width) as response variables, respectively.
220 General Linear Models were also used to test the effect of our experimental
221 manipulation on each metric of oxidative status. Each model included two main factors
222 (pre-refeeding group and refeeding group) and their interaction. To meet normality of
223 residuals and homogeneity of variances, plasma reactive oxygen metabolites and brain
224 superoxide dismutase were both square-root transformed, while brain protein carbonyls
225 were log-transformed. Transformations were chosen according to the fitting of the
226 models using values of Akaike Information Criterion as a reference. Post-hoc tests were
227 used to explore further any significant interaction across logical sets of means. When
228 non-significant, interactions were removed from the models. Outcomes of full and
229 reduced models coincided when there was not backward elimination because the
230 interaction/s was/were significant.

231

232 **3. Results**

233 The difference in body size between control and treated fish depended on whether fish
234 were re-fed on a normal or enriched diet (three-way interaction: $p = 0.047$). To

235 investigate this further, the effect of the experimental manipulation on body size was
236 tested separately for fish re-fed on a normal or enriched diet, using similar linear models
237 as explained above. Fish re-fed on a normal diet did not catch-up, resulting in a smaller
238 body size than those fish that were not food-deprived (Fig. 2, Table 1). Conversely, fish
239 re-fed on an enriched diet were capable to catch-up, resulting in a body size as that of
240 fish that were not food-deprived (Fig. 2, Table 1). Similar results were obtained if body
241 size was extracted from a PCA calculated on body mass and body width (three-way
242 interaction: $p = 0.012$). As for body length, fish that were food-deprived were shorter
243 than fish that were not food-deprived at the end of the food deprivation period, while
244 they were of similar body length at the end of the experiment (Fig. 3; Table 1). The
245 effect of food deprivation was not influenced by the quality of diet given after the food
246 deprivation period (Table 1).

247 The difference in plasma reactive oxygen metabolites between control and
248 treated fish depended on whether fish were re-fed on a normal or enriched diet. Fish that
249 were fed on an enriched diet after food deprivation had significantly higher plasma
250 reactive oxygen metabolites than all other experimental groups (Fig. 4, Table 1).

251 Plasma protein carbonyls were higher in fish that were either fed continuously or
252 re-fed on an enriched diet than those either fed continuously or re-fed on a normal diet
253 (Fig. 5, Table 1). Irrespective of the quality of re-feeding diet, brain protein carbonyls
254 were higher in fish that were food-deprived than in fish that were not (Fig. 6, Table 1).

255 The activity of glutathione peroxidase in red blood cells was higher in fish that
256 were either fed continuously or re-fed on an enriched diet than those either fed
257 continuously or re-fed on a normal diet (Fig. 5, Table 1). The activity of brain

258 glutathione peroxidase was unaffected by the experimental manipulation in both phases
259 (Table 1).

260 The activity of superoxide dismutase in both red blood cells and brain was lower
261 in fish that were either fed continuously or re-fed on an enriched diet than those either
262 fed continuously or re-fed on a normal diet (Fig. 5, Table 1).

263

264 **4. Discussion**

265 The results of this study show that a diet rich in vitamin E may favour compensatory
266 growth after a prolonged period of fasting, but this comes at a cost in terms of increased
267 oxidative damage. As expected, our food deprivation regime slowed down growth of
268 fish. A re-feeding period of six months enabled fish to compensate through a faster
269 growth, but the compensatory growth was mainly evident for mass/condition, while
270 there was not compensation for body length (proxy of skeletal size). There are at least
271 two possible explanations. Previous studies on fish have shown that (i) growth
272 compensation may be limited in some species to restoring lost tissues/energy reserves,
273 with no acceleration of skeletal growth (Alvarez and Nicieza, 2005), or (ii) growth
274 compensation of body length may take longer than body mass/condition to be evident
275 (e.g., Johnsson and Bohlin, 2006).

276 The compensatory growth occurred only in those fish that were re-fed with a
277 diet enriched with vitamin E, highlighting the importance of this nutrient for fish
278 growth. Vitamin E may interact with many metabolic and cellular pathways important
279 for growth, such as lipid transport by lipoproteins, vitamin C synthesis, function of
280 erythrocytes, immunity or metabolism of other nutrients (Hamre, 2011). Previous work
281 on juvenile gilthead seabreams (*Sparus aurata*) also showed that increased intake of

282 vitamin E can reduce skeletal anomalies, favouring growth (Izquierdo et al., 2013).
283 Vitamin E promotes membrane repair and such repair ability is particularly important
284 when cells are being exposed to an oxidant challenge (Howard et al., 2011; Labazi et
285 al., 2015). Moreover, vitamin E may reduce haemolysis of red blood cells through a
286 chain-breaking activity, i.e., able to scavenge free radical by hydrogen donation (Niki et
287 al., 1988; Niki, 2014). Although vitamin E has antioxidant properties, those fish that
288 made a compensatory growth had higher levels of plasma reactive oxygen metabolites
289 than those fish that did not make a compensatory growth. High metabolic activity
290 typical of compensatory growth might have caused a high production of oxygen
291 metabolites. Conversely, protein oxidative damage was not increased in fish that made a
292 compensatory growth. A reason for this might lie with a higher protection of proteins
293 against oxidation. Reactive oxygen metabolites as measured in our work come from
294 early oxidation of biomolecules, and their accumulation is prevented by the activity of
295 the glutathione peroxidase (Halliwell and Gutteridge, 2015). On the other hand, protein
296 carbonylation is mostly irreversible damage, thus avoidance of carbonylation might
297 have been prioritised and traded-off against other kinds of damage.

298 The higher production of reactive oxygen metabolites in those fish that made a
299 compensatory growth might have also been favoured by the impact that vitamin E
300 intake had on antioxidant enzymes. Previous work on fish showed that vitamin E may
301 reduce the expression of superoxide dismutase gene and increase the expression of the
302 glutathione peroxidase gene, respectively (Izquierdo et al., 2013). These results on
303 expression of enzymatic antioxidant genes are in agreement with our results on the
304 activity of superoxide dismutase and glutathione peroxidase. Superoxide dismutase
305 catalyses the dismutation of the superoxide anion generated by mitochondria into

306 oxygen and hydrogen peroxide; glutathione peroxidase reduces hydrogen peroxide and
307 organic hydroperoxides into water and alcohols, respectively (Halliwell and Gutteridge,
308 2015). Hence, lower activity of superoxide dismutase might have resulted in reduced
309 protection against the peroxidative action of superoxide anion, which may increase
310 generation of organic hydroperoxides (Halliwell and Gutteridge, 2015). Upregulation of
311 glutathione peroxidase might have been needed to detoxify cells from organic
312 hydroperoxides, which may propagate the oxidative cascade (Halliwell and Gutteridge,
313 2015). Fish feeding on the diet enriched with vitamin E had actually higher protein
314 oxidative damage than those feeding on a normal diet.

315 Although vitamin E has antioxidant properties, any beneficial effects of having
316 large amounts of vitamin E available do not necessarily come through its antioxidant
317 properties. Selman et al. (2008) showed that supplementation of vitamin E significantly
318 increased median lifespan of mice by 15 % relative to non-supplemented controls and
319 also increased maximum lifespan. Lymphocyte and hepatocyte oxidative DNA damage
320 and hepatic lipid peroxidation were, however, unaffected by supplementation. Using a
321 cDNA macroarray, Selman et al. (2008) showed that genes associated with xenobiotic
322 metabolism were significantly upregulated in the livers of mice, suggesting that vitamin
323 E is initially metabolized by cytochrome P450s in a manner similar to xenobiotics. The
324 absence of any significant effect on oxidative damage suggests that the lifespan
325 extension observed in mice was not due to the antioxidant properties of vitamin E.

326 In various invertebrates (e.g., molluscs, crustaceans) and fish, data on
327 development of antioxidant defences suggested that dietary and other low molecular
328 weight antioxidants are predominant in the earliest stages of development, while
329 antioxidant enzymes become predominant later in life (Rudneva, 1999; Surai, 2002;

330 Fontagné et al., 2008). We do not know if any beneficial effects of dietary antioxidants
331 could have been stronger at earlier stages of development. This is an open question that
332 deserves further work to understand at which developmental stage intake of dietary
333 antioxidants might be more beneficial. It will also be important to assess the amount of
334 tocopherols that can be retained in tissues and which forms of tocopherols and of their
335 metabolites have a stronger impact on the antioxidant machinery during development
336 (Brigelius-Flohé and Traber, 1999; Surai, 2002).

337 Our results also show that experiencing a period of food deprivation may have
338 long-term effects on the protein oxidative damage in brain. Irrespective of the quality of
339 re-feeding diet, all the fish that underwent food restriction had higher oxidative damage
340 in brain later in life than those that did not. Restriction of intake of specific nutrients in
341 early life may have pervasive, long-lasting effects on oxidative status. For example, rats
342 subjected to prenatal and postnatal protein malnutrition had increased oxidative damage
343 to lipids and proteins in brain (Feoli et al., 2006). Zebra finches (*Taeniopygia guttata*)
344 that experienced a short period of low quality nutrition during the nestling period had a
345 twofold reduction in plasma levels of carotenoids, vitamins A and E in adulthood
346 (Blount et al., 2003). In fish, starvation may elicit profound changes in brain energy
347 metabolism in a way similar to that demonstrated in other vertebrates like mammals.
348 For example, when the fasting period is prolonged and glucose becomes less available
349 with time, the brain obtains a portion of its energy from ketone bodies (Hasselbalch et
350 al., 1994; Campbell and Farrel, 2006), which are important generators of protein
351 carbonylation (Halliwell and Gutteridge, 2015). Moreover, during prolonged fasting,
352 animals face increased protein catabolism and body mass loss (McCue, 2010, 2012),
353 which might favour accumulation of protein carbonyls.

354 In conclusion, our work provides evidence that any beneficial effects of dietary
355 antioxidants on growth might be offset by an impaired capability of regulating the
356 oxidative status. If investing more into compensatory growth is more advantageous than
357 into self-maintenance and mechanisms favouring lifespan likely depends on the
358 environmental conditions young develop in, such as predation risk or food availability.
359 Work on oxidative stress resistance under this life-history framework would help to
360 clarify its role as a mediator of some early life tradeoffs across different environments.
361 Our results also point for a role of oxidative stress as a mechanism linking the long-term
362 negative effects of early life poor nutrition on brain function.

363

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369

370 **Competing interests**

371 No competing interests.

372

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376

377 **References**

378 Ali, M., Parvez, S., Pandey, S., Atif, F., Kaur, M., Rehman, H., Raisuddin, S., 2004. Fly
379 ash leachate induces oxidative stress in freshwater fish *Channa punctata* (Bloch).
380 Environm. Intern. 30, 933–938.

381 Alonso-Alvarez, C., Bertrand, S., Faivre, B., Sorci, G., 2007. Increased susceptibility to
382 oxidative damage as a cost of accelerated somatic growth in zebra finches. *Funct.*
383 *Ecol.* 21: 873–879.

384 Alvarez, D., Nicieza, A.G., 2005. Compensatory response ‘defends’ energy levels but
385 not growth trajectories in brown trout, *Salmo trutta* L. *Proc. R. Soc. Lond. B* 272:
386 601–607.

387 Barja, G., 2004. Free radicals and aging. *Trends Neurosc.* 27: 595–600.

388 Birnie-Gauvin, K., Costantini, D., Cooke, S.J., Willmore, W.G., 2017. A comparative
389 and evolutionary approach to oxidative stress in fish: a review. *Fish Fisher.* 18: 928-
390 942.

391 Blanckenhorn, W.U., 2000. The evolution of body size: what keeps organisms small?
392 *Q. Rev. Biol.* 75: 385–407.

393 Blount, J.D., Metcalfe, N.B., Arnold, K.E., Surai, P.F., Devevey, G.L., Monaghan, P.,
394 2003a. Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the
395 zebra finch. *Proc. R. Soc. Lond. B* 270: 1691–1696.

396 Brigelius-Flohé, R., Traber, M.G., 1999. Vitamin E: function and metabolism. *FASEB*
397 *J.* 13: 1145–1155.

398 Campbell, M.K., Farrell, S.O., 2006. *Biochemistry*. 5th ed., Cengage Learning, pp. 579.

399 Careau, V., Bergeron, P., Garant, D., Reale, D., Speakman, J.R., Humphries, M.M.,
400 2013. The energetic and survival costs of growth in free-ranging chipmunks.
401 *Oecologia* 171: 11–23.

402 Costantini, D., 2014. Oxidative stress and hormesis in evolutionary ecology and
403 physiology: a marriage between mechanistic and evolutionary approaches. Springer-
404 Verlag, Berlin Heidelberg, pp. 348.

405 Criscuolo, F., Monaghan, P., Nasir, L., Metcalfe, N.B., 2008. Early nutrition and
406 phenotypic development: “catchup” growth leads to elevated metabolic rate in
407 adulthood. *Proc. R. Soc. Lond. B* 275: 1565–1570.

408 Dmitriew, C.M., 2011. The evolution of growth trajectories: what limits growth rate?
409 *Biol. Rev. Camb. Philos. Soc.* 86: 97–116.

410 Dülger, N., Kumlu, N., Türkmen, S., Olcülü, A., Tufan Eroldoğan, O., Asuman Yilmaz,
411 H., Oçal, N., 2012. Thermal tolerance of European Sea Bass (*Dicentrarchus labrax*)
412 juveniles acclimated to three temperature levels. *J. Therm. Biol.* 37: 79-82.

413 Feoli, A.M., Siqueira, I.R., Almeida, L., Tramontina, A.C., Vanzella, C., Sbaraini, S.,
414 Schweigert, I.D., Netto, C.A., Perry, M.L.S., Gonçalves, C.A., 2006. Effects of
415 protein malnutrition on oxidative status in rat brain. *Nutrition* 22: 160–165.

416 Fontagné, S., Lataillade, E., Brèque, J., Kaushik, S., 2008. Lipid peroxidative stress and
417 antioxidant defence status during ontogeny of rainbow trout (*Oncorhynchus mykiss*).
418 *Br. J. Nutr.* 100: 102–111.

419 Halliwell, B.H., Gutteridge, J.M.C., 2015. Free radicals in biology and medicine. 5th
420 edn, Oxford Univ. Press, Oxford.

421 Hamre, K., 2011. Metabolism, interactions, requirements and functions of vitamin E in
422 fish. *Aquac. Nutr.* 17: 98-115.

423 Hasselbalch, S.G., Knudsen, G.M., Jakobsen, J., Hageman, L.P., Holm, S., Paulson,
424 O.B., 1994. Brain metabolism during short-term starvation in humans. *J. Cerebral*
425 *Blood Flow Metab.* 14: 125-131.

426 Howard, A.C., McNeil, A.K., McNeil, P.L., 2011. Promotion of plasma membrane
427 repair by vitamin E. *Nature Commun.* 2: 597.

428 Izquierdo, M.S., Scolamacchia, M., Betancor, M., Roo, J., Caballero, M.J., Terova, G.,
429 Witten, P.E., 2013. Effects of dietary DHA and α -tocopherol on bone development,
430 early mineralisation and oxidative stress in *Sparus aurata* (Linnaeus, 1758) larvae.
431 *Br. J. Nutr.* 109: 1796-805.

432 Johnsson, J.I., Bohlin, T., 2006. The cost of catching up: increased winter mortality
433 following structural growth compensation in the wild. *Proc. R. Soc. Lond. B* 273:
434 1281-1286.

435 Labazi, M., McNeil, A.K., Kurtz, T., Lee, T.C., Pegg, R.B., Angeli, J.P., Conrad, M.,
436 McNeil, P.L., 2015. The antioxidant requirement for plasma membrane repair in
437 skeletal muscle. *Free Radic. Biol. Med.* 84: 246-253.

438 Lee, W.-S., Monaghan, P., Metcalfe, N., 2016. Perturbations in growth trajectory due to
439 early diet affect age-related deterioration in performance. *Funct. Ecol.* 30: 625-635.

440 Levine, R.L., Garland, D., Oliver, C.N., Amici, A., Climent, I., Lenz, A.G., Ahn, B.W.,
441 Shaltiel, S., Stadtman, E.R., 1990. Determination of carbonyl content in oxidatively
442 modified proteins. *Methods Enzymol.* 186: 464-478.

443 McCue, M.D., 2010. Starvation physiology: reviewing the different strategies animals
444 use to survive a common challenge. *Comp. Biochem. Physiol. Part A* 156: 1–18.

445 McCue, M.D., 2012 *Comparative physiology of fasting, starvation, and food limitation.*
446 Springer-Verlag Berlin Heidelberg.

447 Metcalfe N.B., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later?
448 *Trends Ecol. Evol.* 16: 254–260.

449 Niki, E., Komuro, E., Takahashi, M., Urano, S., Ito, E., Terao, K., 1988. Oxidative
450 hemolysis of erythrocytes and its inhibition by free radical scavengers. *J. Biol.*
451 *Chem.* 263: 19809–19814.

452 Niki, E., 2014. Role of vitamin E as a lipid-soluble peroxy radical scavenger: in vitro
453 and in vivo evidence. *Free Radical Biol. Medic.* 66: 3–12.

454 Nussey, D.H., Pemberton, J.M., Pilkington, J.G., Blount, J.D., 2009. Life history
455 correlates of oxidative damage in a free-living mammal population. *Funct. Ecol.* 23:
456 809–817.

457 Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative
458 characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70: 158-
459 169.

460 Rudneva, I.I., 1999. Antioxidant system of Black Sea animals in early development.
461 *Comp. Biochem. Physiol. Part C* 122: 265–271.

462 Samuels, S.E., Baracos, V.E., 1995. Tissue protein turnover is altered during catch-up
463 growth following *Escherichia coli* infection in weanling rats. *J. Nutr.* 125: 520-530.

464 Selman, C., McLaren, J.S., Meyer, C., Duncan, J.S., Redman, P., Collins, A.R., Duthie,
465 G.G., Speakman, J.R., 2006. Life-long vitamin C supplementation in combination
466 with cold exposure does not affect oxidative damage or lifespan in mice, but
467 decreases expression of antioxidant protection genes. *Mech. Ageing Devel.* 127:
468 897–904.

469 Selman, C., McLaren, J.S., Mayer, C., Duncan, J.S., Collins, A.R., Duthie, G.G.,
470 Redman, P., Speakman, J.R., 2008. Lifelong alpha-tocopherol supplementation
471 increases the median life span of C57BL/6 mice in the cold but has only minor
472 effects on oxidative damage. *Rejuven. Res.* 11: 83–96.

- 473 Smith, S.M., Nager, R.G., Costantini, D., 2016. Meta-analysis indicates that oxidative
474 stress is both a constraint on and a cost of growth. *Ecol. Evol.* 6: 2833–2842.
- 475 Surai, P.F., 2002. Natural antioxidants in avian nutrition and reproduction. Nottingham
476 Univ. Press, Nottingham.

477 Table 1. Outcomes of full and reduced statistical models. FD = Food Deprivation;
 478 POSTFD = diet after the food deprivation. The reduced models were obtained after
 479 backward elimination of non-significant interactions from the full models, starting from
 480 the three-way interaction. Outcomes of full and reduced models coincided when there
 481 was not backward elimination because the interaction/s was/were significant. Markers
 482 of oxidative status were analysed in a subsample of 40 individuals (10 for each group).
 483
 484

Variable	Effect	Full model			Reduced model		
		d.f.	F	P	d.f.	F	p
Body size PC1 – Postdeprivation standard diet	Treatment group	1,147	28.6	<0.001	1,147	28.6	<0.001
	Sampling day	2,147	160.8	<0.001	2,147	160.8	<0.001
	Treatment group × Sampling day	2,147	8.3	<0.001	2,147	8.3	<0.001
Body size PC1 – Postdeprivation enriched diet	Treatment group	1,152	14.1	<0.001	1,152	13.5	<0.001
	Sampling day	2,152	209.2	<0.001	2,152	205.2	<0.001
	Treatment group × Sampling day	2,152	2.9	0.06			
Body length	Treatment group FD	1,289	15.7	<0.001	1,294	15.6	<0.001
	Treatment group POSTFD	1,289	0.05	0.82	1,294	0.06	0.80
	Sampling day	2,289	356.9	<0.001	2,294	357.2	<0.001
	Treatment group FD × sampling day	2,289	4.5	0.012	2,294	4.6	0.011
	Treatment group POSTFD × sampling day	2,289	0.8	0.46			
	Treatment group FD × Treatment group POSTFD	1,289	1.4	0.23			
	Treatment group FD × Treatment group POSTFD × sampling day	2,289	0.9	0.40			
Plasma ROMs	Treatment group FD	1,39	8.1	0.007	1,39	8.1	0.007
	Treatment group POSTFD	1,39	2.6	0.12	1,39	2.6	0.12
	Treatment group FD × Treatment group POSTFD	1,39	16.4	<0.001	1,39	16.4	<0.001
Plasma protein carbonyls	Treatment group FD	1,39	3.7	0.063	1,39	3.7	0.063
	Treatment group POSTFD	1,39	4.5	0.042	1,39	4.4	0.043
	Treatment group FD × Treatment group POSTFD	1,39	1.3	0.26			
Brain protein carbonyls	Treatment group FD	1,39	7.2	0.011	1,39	7.4	0.01
	Treatment group POSTFD	1,39	1.8	0.19	1,39	1.9	0.18
	Treatment group FD × Treatment group POSTFD	1,39	0.01	0.92			
Red blood cell GPX	Treatment group FD	1,39	2.2	0.15	1,39	2.2	0.15
	Treatment group POSTFD	1,39	9.8	0.003	1,39	9.7	0.004
	Treatment group FD × Treatment group POSTFD	1,39	1.4	0.24			
Brain GPX	Treatment group FD	1,39	0.04	0.85	1,39	0.04	0.85
	Treatment group POSTFD	1,39	0.9	0.36	1,39	0.8	0.37
	Treatment group FD × Treatment group POSTFD	1,39	2.5	0.12			
Red blood cell SOD	Treatment group FD	1,39	1.0	0.32	1,39	1.0	0.32

	Treatment group POSTFD	1,39	5.7	0.02	1,39	5.8	0.022
	Treatment group FD x Treatment group POSTFD	1,39	0.9	0.35			
Brain SOD	Treatment group FD	1,39	0.2	0.68	1,39	0.2	0.68
	Treatment group POSTFD	1,39	5.8	0.02	1,39	5.7	0.022
	Treatment group FD x Treatment group POSTFD	1,39	1.6	0.21			

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487 **Figure captions**

488 Figure 1. Experimental setting and timeline.

489

490 Figure 2. Food deprivation caused a slowdown of growth as suggested by the smaller
491 body size compared to fish that were not food-deprived. Refeeding caused accelerated
492 growth only in those fish that were fed on a diet richer in vitamin E.

493

494 Figure 3. Food deprivation caused a slowdown of skeletal growth as suggested by the
495 smaller body length of food-deprived fish compared to fish that were not food-deprived
496 soon after the food deprivation period (Tukey test, $p < 0.01$) and at the end of the re-
497 feeding experiment (Tukey test, $p < 0.01$).

498

499 Figure 4. Fish that made a compensatory growth (black square) had higher plasma
500 oxidative damage (expressed as reactive oxygen metabolites).

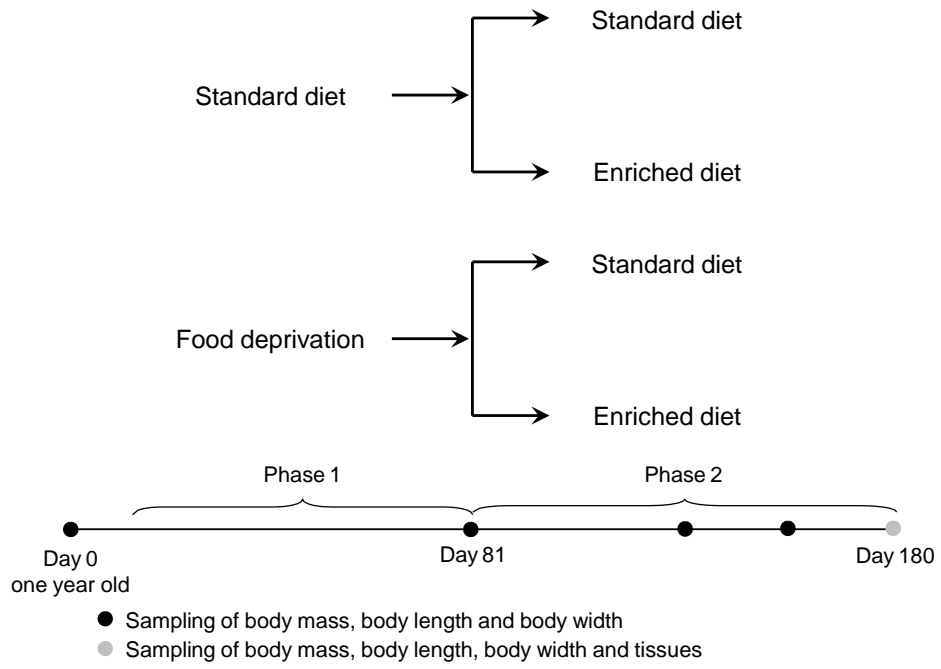
501

502 Figure 5. Fish fed on the diet richer in vitamin E had higher plasma protein oxidative
503 damage (protein carbonyls) and activity of glutathione peroxidase in red blood cells
504 (RBS) and lower activity of superoxide dismutase in both red blood cells and brain.

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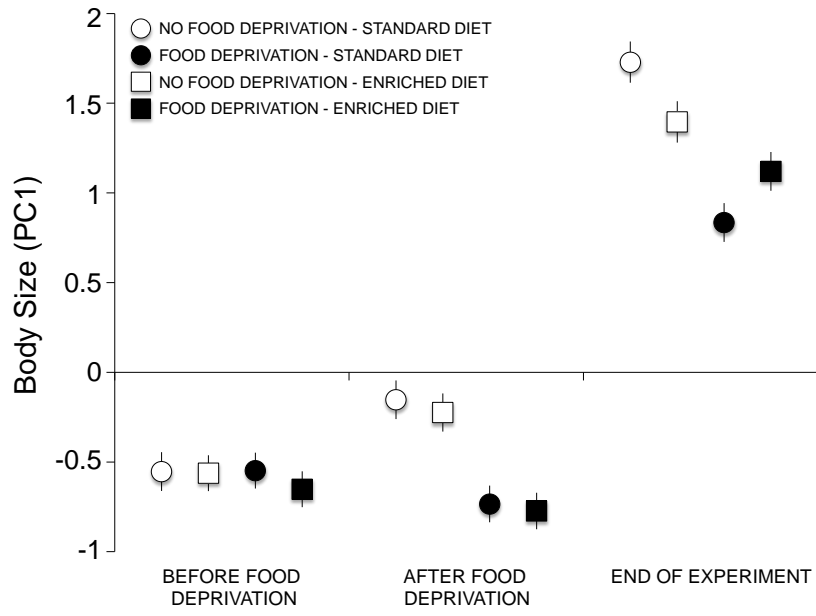
506 Figure 6. Fish that experienced food deprivation had higher brain protein oxidative
507 damage (protein carbonyls) than those fish that were fed ad libitum.

508



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 510 Figure 1
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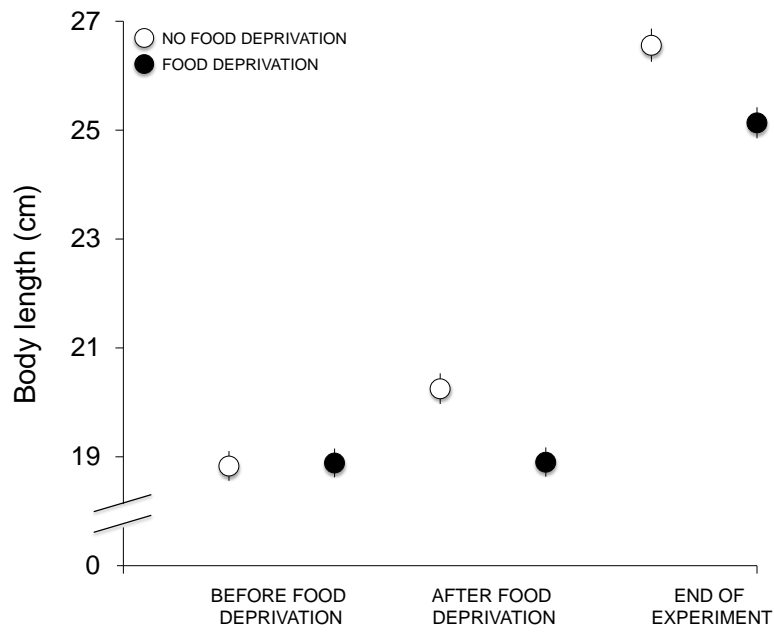


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514 Figure 2

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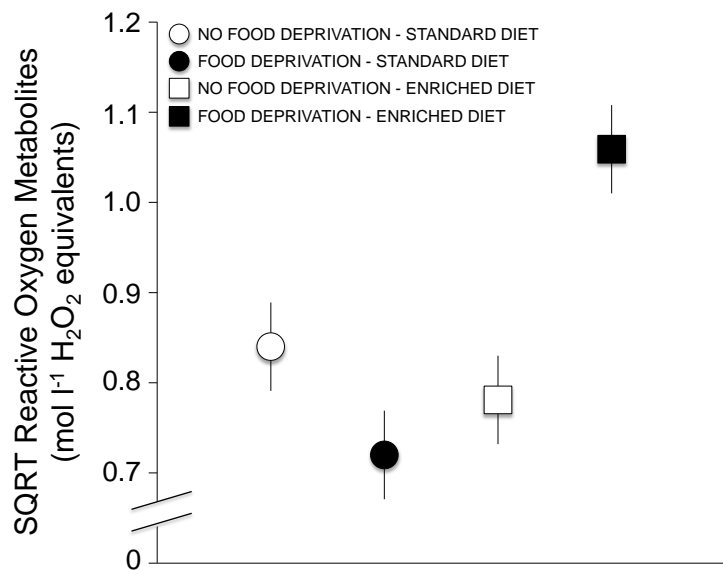


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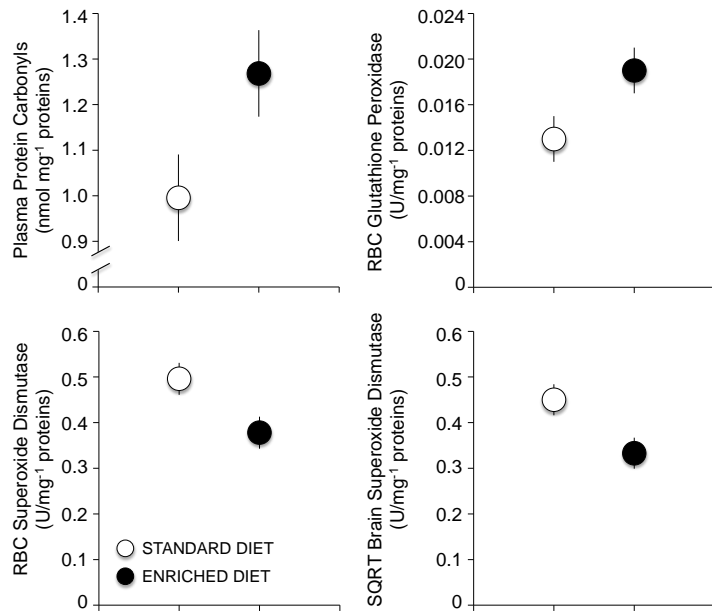
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Figure 3



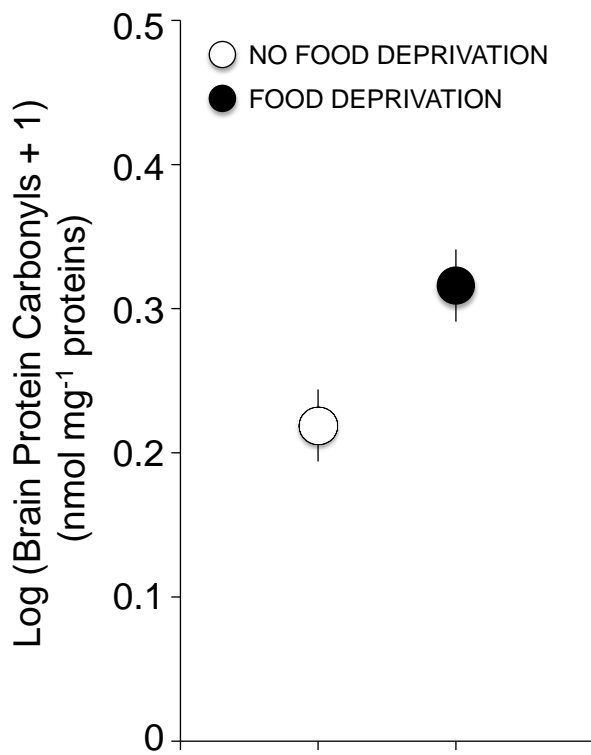
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Figure 4



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Figure 5



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530 Figure 6
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