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1 **Intraspecific variation in freshwater tolerance has consequences for telomere dynamics**  
2 **in the euryhaline teleost *Dicentrarchus labrax***

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9 **freshwater tolerance, cell dynamics, energy metabolism, oxidative stress**

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17

**18 Abstract**

19 Stressful events can alter organism physiology at several levels triggering allostatic responses.  
20 Telomeres are well-conserved repetitive DNA sequences mainly localised at chromosome's  
21 ends, playing a crucial role in DNA stability. Analyses of telomere dynamics are new tools to  
22 assess consequences of environmental stress in non-model organisms like fish. In this study,  
23 the relationship between freshwater tolerance and telomere dynamics was investigated in the  
24 gills of the European sea bass *Dicentrarchus labrax*. Fluorescent *in situ* hybridisation of  
25 telomeric sequences revealed distal telomeres as well as intrachromosomal telomeres known  
26 as interstitial telomere sequences. In order to better understand telomere dynamics in the gills  
27 of *D. labrax*, we used quantitative PCR to measure telomere length and mRNA expression of  
28 the catalytic subunit of telomerase reverse transcriptase *tert*. For the calculation of the relative  
29 telomere length, two reference genes were tested: the single copy gene *mc2r*, encoding  
30 melanocortin 2 receptor and the multicopy gene *18S*, encoding the 18S ribosomal RNA. We  
31 proposed a novel normalisation method to calculate the relative telomere length using both,  
32 single and multiple copy genes as references. Cell dynamics was also investigated by  
33 measuring mRNA expression of genes involved in apoptosis (*caspase 8* and *9*), cell  
34 proliferation (*proliferation cell nuclear antigen*), aerobic mitochondrial metabolism (*ATP*  
35 *citrate-synthase*), anaerobic metabolism (*lactate dehydrogenase a*) and antioxidant enzymatic  
36 defences (*superoxide dismutase 1* and *2*, *catalase*). Following a 15-days fresh water exposure,  
37 telomere dynamics was not significantly modified in the gills of freshwater tolerant fish. But  
38 freshwater intolerant fish exhibited telomere attrition relative to saltwater controls, and lower  
39 expression of *tert* in gills relative to freshwater tolerant fish. This modification of telomere  
40 dynamics in intolerant individuals was found to be correlated with lower antioxidant  
41 enzymatic defences, a higher aerobic metabolic marker and a lower cellular turnover. These  
42 data bring new perspectives for the use of telomere dynamics as an integrative marker to

- 43 study environmental stress in fish, while considering individual phenotypic plasticity in  
44 response to freshwater exposure.

## 45 **Introduction**

46 Marine organisms living in fluctuating environments such as lagoons and estuaries have to  
47 constantly deal with abiotic stressors (salinity, temperature, oxygen). A strong physiological  
48 plasticity is required to be able to face salinity, temperature and oxygen level fluctuations  
49 (Claireaux and Lagardère 1999). The European sea bass *Dicentrarchus labrax* (Linnaeus,  
50 1758) is a demersal fish of high commercial interest which inhabits coastal waters. *D. labrax*  
51 enters estuaries, lagoons and sometimes ascending rivers most likely to feed (Rogdakis et al.  
52 2010). In *D. labrax*, a strong intra-specific variability was highlighted regarding its capacity  
53 to tolerate hyperthermia (Ozolina et al. 2016), hypoxia (Claireaux et al., 2013; Joyce et al.,  
54 2016) and freshwater exposure (Nebel et al., 2005, L'Honoré et al., 2019, 2020), suggesting  
55 an inter-individual difference in the capacity to tolerate harsh environmental conditions.  
56 Burton and Metcalfe (2014) highlighted that exposure to stressful conditions in early life  
57 stages can have long-term and inter-generational effects on physiology and fitness in several  
58 taxa, including fish. It is questionable whether repeated stress encountered throughout life by  
59 fish such as *D. labrax* migrating seasonally in transitional waters, has a negative impact on  
60 fitness.

61 Few studies have examined the potential use of telomere length as an integrative marker of  
62 stress exposure in fish (Anchelin et al. 2013; Henriques et al. 2013; Naslund et al. 2015;  
63 Debes et al. 2016). Telomeres are well conserved terminal regions of eukaryotic  
64 chromosomes, composed of repetitive sequences of TTAGGG in vertebrates (Blackburn and  
65 Gall 1978). Telomeres ensure multiple functions in preserving chromosome stability,  
66 including protecting the ends of chromosomes from degradation and preventing chromosomal  
67 end fusion (Blackburn 1991). Telomere length (TL) and telomerase activity are commonly  
68 used to study ageing in higher vertebrates (Aubert and Lansdorp 2008; Saretzki 2018).  
69 Telomerase plays a crucial role in chromosome stability and cell viability by extending the

70 distal 3' end of eukaryotic linear chromosome over replications (Blackburn 2005). This  
71 enzymatic complex consists of the telomerase reverse transcriptase (TERT) catalytic subunit,  
72 the telomerase RNA component (TERC) involved in the replication of the telomere sequence,  
73 and other associated proteins contributing to elongate telomeres localised at the end of the  
74 chromosomes (Blackburn 2005; Smith et al. 2020). In most non-mammal species such as  
75 birds and fish, telomere dynamics relies on two opposite forces: telomere attrition and  
76 telomere restoration, supported by telomerase. In human, chronic oxidative stress and life  
77 stressors can accelerate telomere attrition by decreasing telomerase activity or *tert* expression  
78 levels (Epel et al. 2004; Houben et al. 2008; Starkweather et al. 2014). In ecological studies,  
79 telomere length provides a mechanistic link between environmental condition, life history  
80 traits and fitness (Monaghan and Haussmann 2006; Haussmann 2010; Monaghan 2014;  
81 Mathur et al. 2016). According to recent meta-analyses focused on ecological studies in non-  
82 model vertebrates (Angelier et al. 2018; McLennan et al. 2018; Wilbourn et al. 2018), we still  
83 lack crucial basic data to fully understand: (i) the influence of abiotic factors, such as salinity  
84 or temperature, on telomere length, (ii) the intra-specific variation in telomere dynamics and  
85 the drivers of this intra-specific variation and (iii) the potential link between telomere  
86 attrition, lifespan and mortality risk, especially in bony fish species.

87 The effect of temperature on telomere attrition was the main environmental abiotic parameter  
88 analysed in fish. In mosquitofish *Gambusia holbrooki*, a decrease from 25°C to 20°C for 24 h  
89 was associated with a decrease in telomere length (Rollings et al. 2014). Conversely, an  
90 increase in temperature from 20°C to 30°C for 1 month triggered telomere attrition in the  
91 Siberian sturgeon *Acipenser baerii* (Simide et al. 2016). Regarding the relationship between  
92 telomere attrition and ageing in fish, studies are controversial. In the zebrafish, telomere  
93 length has been observed to increase from larvae to adult stages and to shorten significantly in  
94 older individuals (Anchelin et al., 2011). Additionally, Hatakeyama et al. (2016) showed in

95 the medaka that telomeres do not shorten linearly with age, but shortening dynamics depends  
96 on growth rate and level of telomerase activity at each life stage. Therefore, it appears that  
97 telomere dynamics is particularly variable and nonlinear in fish.

98 Previous experimental studies performed in juvenile *D. labrax* at different ages have shown  
99 that about 25 to 30% of individuals are unable to acclimate successfully to experimental  
100 transfer from seawater to fresh water (Nebel et al. 2005; L'Honoré et al. 2019, 2020). The  
101 freshwater intolerant phenotype exhibits several characteristics: failure in hydromineral  
102 balance regulation, decrease in swimming capacities, downregulation of gluco- and mineralo-  
103 corticoid receptors involved in both stress response and osmoregulation and, ultimately, death  
104 (Nebel et al., 2005, L'Honoré et al., 2019, 2020). Recently, Angelier et al. (2018) raised new  
105 hypotheses suggesting a trade-off between immediate survival and telomere  
106 maintenance/protection, which would transitionally lead to shortened telomeres during an  
107 “emergency state”. In this study, we compare extreme phenotypes regarding freshwater  
108 tolerance (tolerant vs intolerant) in order to determine if *D. labrax* exhibiting contrasted  
109 freshwater tolerance differ in telomere dynamics.

110 The gill was considered as a somatic tissue of interest to study the relationship between hypo-  
111 osmotic stress and telomere dynamics as the branchial epithelium exhibits a rapid cell  
112 turnover and a strong morphological plasticity (Nilsson 2007; Kang et al. 2013). In *D. labrax* ,  
113 gills are able to remodel within 1 to 2 weeks in response to fluctuations of environmental  
114 factors like salinity, oxygen availability and temperature (Sollid and Nilsson 2006; Lorin-  
115 Nebel et al. 2006; Nilsson et al. 2012; Masroor et al. 2018). Such plasticity in the response to  
116 environmental change has been demonstrated to be associated to elevated cellular dynamics,  
117 such as cell renewal and apoptosis (Sollid 2005; Tzaneva et al. 2014; Sales et al. 2017;  
118 Mierzwa et al. 2020). In addition, an increased number of gill mitochondrion-rich cells  
119 (MRCs) has been shown in hypo-osmotic environments in numerous species including *D.*

120 *labrax* (Nebel et al. 2005; Masroor et al. 2018), suggesting a raise of energetic demand to fuel  
121 active ion transport (Evans et al. 2005). Interestingly, freshwater intolerant *D. labrax* were  
122 previously characterised by a higher density of branchial MRCs compared to freshwater  
123 tolerant fish (Nebel et al. 2005), suggesting metabolic disorders in freshwater intolerant *D.*  
124 *labrax* .

125 Since mitochondria are known to be the main source of ROS production in cells (Lambert and  
126 Brand 2009), an increase in mitochondria may also trigger an increased production of  
127 metabolic ROS, as a by-product of cellular respiration (Quijano et al. 2016). *In vitro*,  
128 oxidative stress was shown to be a major factor triggering DNA damage and accelerated  
129 telomere shortening in human endothelial cells, through the reduction of telomerase activity  
130 (Kurz et al. 2004; Ahmed and Lingner 2017). *In vivo* studies showing a direct link between  
131 oxidative stress and telomere dynamics are more scarce (Boonekamp et al. 2017). Recent  
132 reviews by Reichert & Stier (2017) and Chatelain et al (2019) concluded that there is strong  
133 evidence from both experimental and correlative *in vivo* studies in vertebrates that oxidative  
134 stress induces effects on telomere dynamics, with tissue-dependent, life stage-dependant and  
135 sex-dependant variations. Nevertheless, more experimental studies are required to further  
136 understand the influence of oxidative stress on telomere dynamics *in vivo*.

137 The first aim of this study was to determine the occurrence and the localisation of telomeres  
138 in *D. labrax* genome using fluorescence *in situ* hybridisation (FISH) in order to test if  
139 interstitial telomeric sites are detected. The head kidney was used for karyotyping because of  
140 its high cell renewal (Bertollo et al. 2015). Then, an acute 2 weeks freshwater stress was used  
141 to test whether osmotic stress affects telomere dynamics in the gills of 5-month-old *D. labrax*  
142 exhibiting contrasted freshwater tolerance capacities, as previously described in L'Honoré *et*  
143 *al.* (2019). Telomere attrition was evaluated using relative TL measurement using q-PCR and  
144 the mRNA expression of *tert* was measured as a proxy of telomere maintenance. To better



145 understand cell dynamics and the potential influence of oxidative stress and energy  
146 metabolism on telomere dynamics, mRNA expression of genes involved in apoptosis  
147 (*caspase 8* and *9*), cell proliferation (*proliferation cell nuclear antigen*), aerobic mitochondrial  
148 metabolism (*ATP citrate-synthase*), anaerobic metabolism (*lactate dehydrogenase a*) and  
149 antioxidant enzymatic defences (*superoxide dismutase 1* and *2*, *catalase*) were measured.  
150 Osmotic stress and individual tolerance to fresh water may differentially influence telomere  
151 dynamics where telomere attrition would reflect the harshness of the environment an  
152 individual has experienced. We hypothesised that non-tolerant fish to fresh water will exhibit  
153 shorter telomeres than tolerant fish, as a consequence of oxidative and physiological stress. If  
154 telomeres shorten to critical levels in the gill tissue, this may trigger organ dysfunction, as  
155 previously shown in gut and muscle zebrafish (Carneiro et al. 2016). This could have  
156 consequence on general physiology and survival since the fish gill is a multifunctional organ  
157 involved in gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous  
158 waste (Evans et al. 2005). As short telomeres induce senescence in cells and hence reduce the  
159 regenerative capacity of the corresponding tissues, it has been suggested that TL might affect  
160 various fitness parameters (Monaghan and Haussmann 2006). In fact, TL has been linked to  
161 survival and reproductive success in some bird species (Haussmann et al. 2005; Pauliny et al.  
162 2006). From an evolutionary ecology point of view, telomere-induced selection could occur if  
163 telomere attrition differently affects relative fitness among individuals (Olsson et al. 2017).  
164 Evidence for causal effects of telomere traits on life history and fitness-related parameters is  
165 still limited. In this study, we investigate the consequence of an acute osmotic stress in the  
166 non-model euryhaline species *D. labrax* to test whether intraspecific differences in  
167 osmoregulatory capacities have consequences on TL maintenance.

168

## 169 **Materials and methods**

170 1. Origin of animals

171 Fish were issued from *in vitro* fertilisation of unrelated wild native West Mediterranean  
172 breeders (40 males and 23 females) in order to obtain a large genetic diversity. Sea bass were  
173 grown at the Ifremer Station at Palavas-les-flots (Hérault, France) under a 16/8 hours  
174 light/dark photoperiod in seawater (SW) at 20°C. Food was proposed *ad libidum*.

175 2. Fluorescence *in situ* hybridisation on telomere sequence DNA and microscope  
176 analysis

177 Karyotype analysis and fluorescent *in situ* hybridisation were performed at the CytoEvol  
178 facilities of UMR ISEM of the LabEx CeMEB (Montpellier, France). Cephalic kidney of two  
179 males and 2 females (10 month-old) were sampled and processed as described in Ozouf-  
180 Costaz *et al.* (2015). Fluorescence *in situ* hybridisation (FISH) was performed following the  
181 same procedure as described in Ozouf-Costaz *et al.* (2015), using an oligonucleotide  
182 telomeric probe (TTAGGG)<sub>7</sub> labelled with Cy3 at its 5' end (biomers.net, Ulm, Germany) and  
183 counterstaining the chromosomes with DAPI (4',6-diamidino-2-phenylindole)-antifade  
184 mounting medium solution (Vectashield, Vector Laboratories, Peterborough, UK). Three  
185 slides were prepared per individual and preparations were analysed using a Zeiss Axioplan 2  
186 Imaging epifluorescence microscope equipped with a cooled charge couple devise camera and  
187 Cytovision 7.4 software (Applied Imaging, San Jose, CA).

188 3. Experimental exposure to freshwater

189 Five month-old *D. labrax* juveniles (N=1525, 4.20 ± 0.09 cm, 0.87 ± 0.06 g) were  
190 experimentally exposed to fresh water according to L'Honoré *et al.* (2019). Briefly, fish were  
191 transferred from SW to brackish water (BW) at 15 ppt for 24h before being transferred to  
192 fresh water (FW) for 2 weeks. A no replication experimental setup, where intolerant fish and  
193 tolerant fish are maintained in the same tank and exact same conditions, was chosen because

194 we expected from previous studies that the FW intolerant phenotype represents about one  
195 third of the experimental cohort (Nebel et al. 2005; L'Honoré et al. 2019, 2020), thus  
196 requiring an elevated number of animals (N=1525). In addition, the detection of FW  
197 intolerant phenotype also requires an elevated number of individuals swimming in shoals in  
198 order to be able to observe abnormal individual behaviour within the shoal as described in  
199 L'Honoré et al. (2019). After 2 weeks of freshwater challenge, tolerant and intolerant  
200 phenotypes were sorted, measured and weighted. More precisely, fish exhibiting erratic  
201 swimming, isolation from the shoal associated with low reflexes and stronger pigmentation  
202 were identified as the freshwater-intolerant phenotype (FW-I). These animals were  
203 characterised by an incapacity to maintain hydromineral balance in FW (Nebel et al., 2005;  
204 L'Honoré et al. 2019, 2020). The three experimental groups analysed were: seawater controls  
205 (SW,  $6.30 \pm 0.12$  cm,  $2.84 \pm 0.14$  g), freshwater tolerant fish (FW-T,  $5.28 \pm 0.10$  cm,  $1.47 \pm$   
206  $0.10$  g) and freshwater intolerant fish (FW-I,  $5.20 \pm 0.10$  cm,  $1.25 \pm 0.08$  g). At the end of the  
207 exposure, fish were euthanised in 100 ppm of benzocaine and the first left gill arc was  
208 dissected, flash frozen in liquid nitrogen and stored respectively dry or in *RNAlater* (Quiagen,  
209 Valencia, CA) at  $-80^{\circ}\text{C}$  until gDNA and mRNA extraction.

#### 210 4. gDNA extraction

211 Genomic DNA (gDNA) extraction was performed using the Maxwell<sup>®</sup> 16 Buccal Swab LEV  
212 DNA Purification Kit (Promega, Charbonnières, France). Samples were eluted in 50  $\mu\text{L}$  of  
213 ultrapure water. Quantity was measured fluorometrically using a Qubit dsDNA BR Assay Kit  
214 (Thermo Fisher Scientific), concentrations ranged from 60 to 200  $\mu\text{g mL}^{-1}$ . Purity was  
215 verified using the NanoDrop<sup>™</sup> One/One<sup>C</sup> Spectrophotometer (Thermo Scientific, Waltham,  
216 MA, USA) through A260/A280 and A260/A230 ratios. DNA quality was checked using  
217 Bioanalyzer 2100 (Santa Clara, CA, United States).

## 218 5. RNA extraction and reverse transcription

219 RNA extraction was performed using the total RNA extraction kit that includes a DNase step  
220 (Nucleospin<sup>®</sup> RNA, Macherey-Nagel, Germany). Quantity and purity of extraction products  
221 were verified using a UV spectrophotometer (NanoDrop<sup>™</sup> One/OneC Spectrophotometer,  
222 Thermo Scientific, Waltham, MA, USA). RNA quality was checked using Bioanalyzer 2100  
223 and RIN levels were comprised between 6 and 9 (mean RIN = 8.15). Reverse transcription  
224 was performed using one microgram of RNA using the qScript<sup>™</sup> cDNA SuperMix (Quanta  
225 Biosciences<sup>™</sup>) providing all necessary components for first-strand synthesis: buffer,  
226 oligo(dT) primers, random primers and qScript reverse transcriptase.

## 227 6. Target genes selection

228 Key genes were selected to better understand cell dynamics and the potential influence of  
229 oxidative stress and energy metabolism on telomere dynamics. Proliferating cell nuclear  
230 antigen (*pcna*) was used as a cell proliferation marker (Sadoul et al. 2018) whereas caspase 8  
231 and 9 (*casp 8*, *casp 9*) were used as extrinsic and intrinsic cell apoptosis markers respectively  
232 (Olsson and Zhivotovsky 2011; Paiola et al. 2018). Mitochondrial superoxide dismutase 1  
233 (*sod1*), cytosolic superoxide dismutase 2 (*sod2*) and catalase (*cat*) were selected as  
234 antioxidant enzymes because superoxide anion ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ) are the  
235 main ROS formed by mitochondria. Lactate dehydrogenase is a key enzyme in the control of  
236 energy metabolism composed of four polypeptide subunits encoded by two genes: *ldh-a* and  
237 *ldh-b* (Driedzic et al. 1980). In this study, *ldh-a* was investigated as a marker for anaerobic  
238 glycolysis (Almeida-Val et al. 2011; Valvona et al. 2016). Gene encoding citrate synthase (*cs*)  
239 was selected as a marker of aerobic metabolism (Roche and Reed 1974; Elcock and  
240 McCammon 1996; Goldenthal et al. 1998).

## 241 7. Quantitative real-time polymerase chain reaction

242 Telomere length measurement and relative mRNA gene expression quantification was  
243 realised using 384-wells plates filled with an Echo®525 liquid handling system (Labcyte Inc.,  
244 San Jose, CA, USA). Each well contained a mix composed by 1.5 µL of LightCycler-  
245 FastStart DNA Master SYBR-Green I™ Mix (Roche, Mannheim, Germany), 0.27 µL of each  
246 primer (forward and reverse primers at 0.9 µM final concentration), 0.23 µL of ultrapure  
247 water and 1 µL of cDNA or gDNA. For *pcna*, *tert*, *casp8*, *casp9*, *sod1*, *sod2*, *cat*, *cs*, *ldh-a*,  
248 *18S* and *113* cDNA amplification, efficiency (E) of each primer pair was tested using standard  
249 curves performed on all-samples pools of cDNA (Table 1).

250 For mRNA expression analyses, the q-PCR conditions were as follows: 2 min denaturation at  
251 95 °C followed by 35 cycles (95 °C for 30 s, 61 °C for 45 s and 72 °C for 1 min) followed by  
252 a final elongation step at 72 °C for 4 min. The reference genes *18S* and *113* were chosen  
253 according to previous studies performed in sea bass (Mitter et al. 2009). Relative mRNA  
254 expressions were normalised against two reference genes, *113* and *18S*, according to the  
255 method of Vandesompele et al. (2002) and expressed using the comparative  $\Delta\Delta C_t$  method (Ct,  
256 threshold cycle number) described by Pfaffl (2001), with SW fish as a reference. For all  
257 samples, measurements were run in triplicates, and no-template control (water) Ct was above  
258 40.

259 For TL measurement, the q-PCR conditions were adapted from Cawthon (2009) with some  
260 modifications as follows: 15 min denaturation at 95 °C followed by 2 cycles (94° C for 15 s,  
261 49° C for 15 s) followed by 35 cycles (95 °C for 15 s, 62 °C for 10 s and 74 °C for 15 sec).  
262 Telomere primers used to amplify telomeric hexamer repeats were TEL G and TEL C as  
263 described in Cawthon (2009). Efficiency of each primer pairs reported on Table 1 were  
264 obtained by standard curves performed on all-sample pools of gDNA (*tel*, *mc2r* and *18S*).  
265 Relative TL calculation was performed using the ratio between telomere repeat copy number  
266 and reference gene copy number known as T/R ratio. T/R ratio was calculated with the  $\Delta\Delta C_t$

267 method described in Cawthon (2002) and normalised against two reference genes, a single  
268 copy gene *mc2r* and a multicopy gene *18S* (Wang et al. 2013).

269 Formulas used “E” as primers efficiency as indicated in Table 1. The condition SW was used  
270 as the control condition for the  $\Delta\Delta\text{Ct}$  calculation. An inter-plate assay was performed to  
271 investigate the potential variability between two different q-PCR runs. Inter-assay validation  
272 was performed in duplicates with *mc2r* and *18S* on gDNA of 16 samples.

## 273 8. Statistics

274 Statistical analyses were performed on GraphPad Prism (version 6, GraphPad Software  
275 Incorporated, La Jolla, CA 268, USA). First, Grubb’s test was used on the 15 fish per  
276 condition to remove the potential outliers from the data set. Since data fitted normality test  
277 (D’Agostino-Pearson test) but not homoscedasticity test (Bartlett test), Mann-Whitney  
278 pairwise comparisons were performed, with Bonferroni adjustment ( $p < 0.0167$ ). For inter-  
279 plate assay correlation analysis, Pearson correlation tests were used because data fitted with  
280 normality assumption. A non-parametric Spearman correlation test was performed to study  
281 the correlation between quantitative variables. Experimental values are reported as means  $\pm$   
282 s.e.m..

## 283 Results

### 284 1. FISH of DNA telomere sequences in *D. labrax* karyotypes

285 Karyotype analyses confirmed the presence of  $2n=48$  chromosomes (Fig. 1) as expected in *D.*  
286 *labrax* (Sola et al., 1993). Fluorescent in situ hybridisation of telomeric sites revealed that  
287 telomere sequences were localised distally, as expected. Interstitial telomeric sequences (ITS)  
288 localised proximally were also observed. FISH does not allow a precise detection so we

289 cannot conclude about any inter-individual differences in signal intensity or localisation of  
290 telomere sequences.

## 291 2. Relative telomere length measurements

### 292 2.1 Method validation

293 Primer efficiency of the single copy gene *mc2r* and the multicopy gene *18S* were at 2.0 (Table  
294 1). The primers specificity was checked using the melting point ( $T_m$ ) of the product for each  
295 primer pair and displayed a unique pike at the expected temperature. The inter-plate Pearson  
296 correlation  $r^2$  were respectively above 0.92 and 0.99 for the T/R ratio with *mc2r* and *18S* as  
297 reference genes (Pearson test,  $P < 0.0001$  for each gene, Figs 1Sa-b). Coefficients of variation  
298 (CV) did not exhibited values  $> 3\%$  for both intra-assay CV and inter-assay CV as resumed in  
299 Table 1S.

300 Regarding TL, CV were the highest using *18S* as the reference gene (32.0% in SW, 34.7% in  
301 FW-T and 60.0% in FW-I, Fig. 1Sc), whereas they were the lowest using *mc2r* as the  
302 reference gene (26.6% in SW, 35.5% in FW-T and 32.0% in FW-I, Figure 2). Thus, we will  
303 consider *mc2r* as the best reference gene for TL since it was 3-times less variable within  
304 phenotypes.

### 305 2.2 Telomere dynamics in response to freshwater exposure: telomere length and 306 mRNA expression of *tert*

307 No significant difference in TL could be measured between SW and FW-T ( $P = 0.8107$ , Fig.  
308 2). A significantly lower relative TL was measured in FW-I compared to FW-T and SW ( $P <$   
309  $0.0001$ , Fig. 2). Regarding *tert* expression, no significant difference was measured between  
310 SW and FW-T ( $P = 0.2115$ , Fig. 3a). In FW-I, *tert* expression was significantly lower than in  
311 FW-T but not compared to SW ( $P = 0.0011$  and  $P = 0.072$  respectively).

## 312 2.3 mRNA expression of genes involved in cell dynamics, metabolism and antioxidant 313 defences

314 Transcript levels of *pcna* did not differ between SW and FW-T ( $P = 0.9144$ ), whereas they  
315 were significantly lower in FW-I than in SW and in FW-T ( $P = 0.0001$  and  $P = 0.0003$ , Fig.  
316 3b). Although we did not measure any significant difference in *casp8* expression levels  
317 between SW and FW-T ( $P = 0.1936$ , Fig. 3c), we measured significant lower expression of  
318 *casp8* in FW-I compared to FW-T but not compared to SW ( $P = 0.0137$  and  $P = 0.0367$   
319 respectively). We did not measure any significant difference in *casp9* expression levels  
320 between the three groups (Fig. 4d).

321 Superoxide dismutase *sod1* and *sod2* mRNA gene expression did not exhibit any significant  
322 differences between the three phenotypes (Figs 4a-b). Regarding *cat*, no significant  
323 differences were inferred between SW and FW-T ( $P = 0.0455$ ). However, FW-I displayed  
324 significantly lower expression compared to both SW and FW-T ( $P = 0.0005$  and  $P = 0.0052$   
325 respectively, Fig. 4c).

326 Concerning *cs* mRNA expression levels, they were significantly lower in SW than in FW-T  
327 and FW-I ( $P = 0.0133$  and  $P = 0.0101$ , Fig. 4d). However, no significant differences could be  
328 inferred between FW-T and FW-I ( $P = 0.6932$ ), or between each group regarding *ldh-a*  
329 mRNA expression levels ( $P = 0.0469$  for SW vs FW-T,  $P = 0.0219$  for SW vs FW-I, and  $P =$   
330  $0.3669$  for FW-T vs FW-I).

## 331 3. Correlation between variables

332 Testing the Spearman coefficient correlation between all quantitative variables (Table 2), it  
333 appeared that telomere dynamics markers (TL and *tert* mRNA expression) were significantly  
334 and positively correlated ( $r = 0.48$ ,  $P = 0.013$ ). Body mass and body length were not  
335 correlated with telomere dynamics markers ( $P > 0.05$  for both), and no significant differences



336 could be inferred between the two phenotypes in FW (Mann-Whitney test,  $P = 0.3110$  and  
337 unpaired t-test,  $P = 0.5893$ , for body mass and body length respectively). These correlations  
338 were all positives regarding cellular turnover markers ( $r = 0.48$  between *pcna* and TL,  $r =$   
339  $0.38$  between *casp8* and TL,  $r = 0.64$  between *casp8* and *tert*,  $r = 0.47$  between *casp9* and  
340 *tert*) and antioxidant enzymatic defences ( $r = 0.66$  between *sod2* and *tert*,  $r = 0.59$  between  
341 TL and *cat*). However, the correlation between TL and metabolic marker was negative ( $r = -$   
342  $0.58$  between *cs* and TL,  $r = -0.39$  between *ldh-a* and TL).

## 343 Discussion

### 344 1. Method validation

345 As reviewed in Lai *et al.* (2018), the estimation of relative TL using the q-PCR method may  
346 be biased by inter-assays variations. By reproducing the same q-PCR amplification using two  
347 different plates as described in Appleby (2016), we showed that the operational variability  
348 was very limited in this study. Karyotype analysis of *D. labrax* revealed 24 pairs of  
349 chromosomes different in size as already demonstrated in the literature (Sola *et al.* 1993). In  
350 this study, we demonstrate the presence of interstitial telomeric sequences (ITS) in *D. labrax*.  
351 According to Ocalewicz *et al.* (2013), most of the pericentromeric and ITS in fish are possible  
352 relicts of chromosome fusion events. The occurrence of ITS may potentially reduce the  
353 sensitivity of the q-PCR method for TL measurement by adding a background noise,  
354 especially if small TL changes are expected. Due to their intrachromosomal position, several  
355 authors suggested that ITS do not shorten during DNA replication or in response to ageing or  
356 stress (Foote *et al.* 2013). According to a recent meta-analysis of Chatelain *et al.* (2020), the  
357 noise in telomere length resulting from interstitial repeats may not mask the differences in the  
358 length of end-cap telomeres between individuals, using the qPCR method or the TRF method.  
359 It would be interesting to use a quantitative technique such as Q-FISH (Lai *et al.* 2018) to

360 further explore the proportion of ITS vs terminal telomeric sequences and to determine  
361 whether the inter-individual variability of ITS is elevated in sea bass.

362 For relative quantification, the choice of the reference gene may be crucial to improve the  
363 reliability of the T/R ratio calculation. While most studies used single copy genes as reference  
364 for relative TL calculation (Lai et al., 2018), Wang et al. (2013) demonstrated in single cells  
365 that a multicopy gene like *18S* was more robust for this calculation. However, given that the  
366 variability among each group seemed to depend on the reference gene, we propose to use a  
367 single copy gene like *mc2r* to reduce bias due to the variations of a single specific reference  
368 gene.

## 369 2. Telomere and cell dynamics in gills following FW exposure

370 The use of TL as a biomarker for environmental stress exposure requires a tissue with active  
371 telomere dynamics. Previous study working on erythrocytes reported no difference in  
372 telomere length in *D. labrax* with age (Horn et al. 2008). Gill tissue has several interesting  
373 properties: a strong plasticity associated with high cell renewal and active cell division  
374 (Nilsson 2007; Tzaneva et al. 2014), the presence of MRCs suggesting an active cellular  
375 respiration and potential increased production of metabolic ROS by-products (Hwang and Lee  
376 2007). To our knowledge, the effect of salinity change on telomere dynamics has never been  
377 studied in euryhaline teleost. In this study, we were able to detect a significant TL reduction  
378 of about 50% in the gill of FW-I after only two weeks of freshwater stress. A strong inter-  
379 individual variability in TL was observed, as expected in vertebrates (Dugdale and  
380 Richardson 2018; Toupance et al. 2019). This highlights that telomere dynamics in gill is  
381 quickly modified. In accordance to the hypothesis of Angelier et al (2019), TL was not  
382 maintained in fish whose survival is threatened, suggesting a trade-off between immediate  
383 survival and telomere protection.

384 Interestingly, the quick telomere attrition measured in FW-intolerant fish was correlated to a  
385 significant lower *tert* expression compared to FW-tolerant fish, suggesting an altered capacity  
386 to maintain TL in intolerant fish facing freshwater stress. Conversely, transfer from seawater  
387 to fresh water did not trigger any significant change in *tert* expression and TL in FW<sub>T</sub>. In  
388 European hake *Merluccius merluccius* and in Atlantic cod *Gadus morhua*, *tert* expression was  
389 found higher in early developmental stages suggesting an higher telomerase demand possibly  
390 linked with elevated tissue renewal and long-term cell proliferation capacity maintenance  
391 (López de Abechuco et al. 2014). However, there is no clear trend concerning the relationship  
392 between ageing and telomerase activity (Hatakeyama et al. 2008; Henriques et al. 2013;  
393 Saretzki 2018). Regarding cell dynamics, freshwater transfer is expected to increase cell  
394 population renewal associated with branchial epithelium remodelling occurring during hypo-  
395 osmotic acclimation (Nilsson 2007; Masroor et al. 2018). We observed no significant changes  
396 in mRNA expression of cell dynamics markers of apoptosis *casp8*, *casp9* or proliferation  
397 *pcna* in FW-T after 2 weeks of exposure. Most of the cellular changes have probably been  
398 completed in successfully acclimated *D. labrax* within 2 weeks of freshwater exposure (Nebel  
399 et al. 2005). In the gill of FW-I, *casp8* and *pcna* levels were significantly down-regulated  
400 compared to the freshwater tolerant condition, suggesting a slowdown of cell dynamics in the  
401 gills of intolerant fish. This is consistent with the results of Carneiro et al. (2016), which  
402 observed a decreased cell proliferation in the gut and testis of *tert*<sup>-/-</sup> mutants zebrafish using  
403 PCNA immunostaining. Conversely, in cellular *in vitro* models, a link between *tert*  
404 overexpression, cell survival and increased cell proliferation has been shown (Dagarag et al.  
405 2004; Aubert and Lansdorp 2008). Given that we highlighted a correlation between cellular  
406 dynamics and telomere dynamics in *D. labrax*, we can hypothesise that the reduction of  
407 cellular dynamics observed in FW-I may be associated to a reduction of *tert* expression or  
408 telomerase activity. The reduction of cell dynamics in intolerant fish may be possibly due to

409 an exhaustion of energetic reserves allocated to osmoregulatory processes. Due to technical  
410 and ethical limitations regarding the number of individuals used for this study, no replication  
411 of freshwater and seawater treatment was performed. Therefore, we cannot exclude a batch  
412 effect between SW and FW fish or other confounding factor that may influence within salinity  
413 treatment response.

### 414 3. Metabolism and antioxidant defences following freshwater exposure

415 Freshwater exposure differentially affected energy metabolism and antioxidant enzymatic  
416 defences in *D. labrax* according to their individual freshwater tolerance capacity. Freshwater  
417 exposure significantly increased mRNA gene expression of the citrate synthase gene, a  
418 marker of aerobic metabolism. Acclimation of teleosts to different environmental salinities  
419 causes depletion of energy which is used to regulate the functioning of various highly energy-  
420 consuming pumps and ion transporters in gill MRCs (Chang et al. 2007; Hwang and Lee  
421 2007; Tseng and Hwang 2008). In tilapia gill epithelial cells, Tseng et al. (2008) have shown  
422 that citrate synthase and LDH proteins were induced after transfer from FW to SW,  
423 confirming the active role of these enzymes to fuel active ion-pumping and fish  
424 osmoregulation. During salinity challenges either from SW to FW or from FW to SW, an  
425 increase in lactate contents and LDH activities has been reported in the gills of several  
426 euryhaline teleost fish (Vijayan et al. 1996; Polakof et al. 2006; Tseng et al. 2008) indicating  
427 the involvement of monocarboxylate metabolites in gill energy consumption during  
428 osmoregulation. In this study, no significant changes in mRNA expression of *ldh-a* could be  
429 inferred according to the Bonferonni adjusted *p*-value of 0.0169. Present mRNA gene  
430 expression data should be taken with caution since they do not reflect the concentration and/or  
431 activity of the related protein. Therefore, additional biochemical analyses (e.g. activity of key  
432 enzymes of aerobic and anaerobic metabolic pathways such as LDH, citrate synthase or  
433 citrate oxidase) would be necessary to confirm the hypothesis of a metabolic distress in FW-

434 intolerant fish. But data from this and previous studies (Nebel et al. 2005; L'Honoré et al.  
435 2019, 2020) converge to this hypothesis. After two weeks in FW, the cost of acclimation is  
436 maintained elevated in FW-I compared to FW-T and SW. This is consistent with results of  
437 previous studies in sea bass showing that (i) intolerant fish over-absorbed ions in the gills to  
438 compensate a renal failure (L'Honoré et al., 2020), (ii) intolerant fish exhibit and  
439 overabundance of MRCs in gills (Nebel et al. 2005) and (iii) intolerant fish exhibit a change  
440 in gluco- and mineralocorticoids regulatory pathways, underlying impairment of hydro-  
441 mineral balance and stress response regulation (L'Honoré et al., 2020). Thus, in species  
442 exhibiting intraspecific variability in abiotic stress tolerance such as salinity in killifish  
443 *Fundulus heteroclitus* (Scott and Schulte 2005), temperature (Ozolina et al. 2016) or hypoxia  
444 (Joyce et al. 2016) in *D. labrax*, differences in gill TL should be further investigated to test  
445 whether differential patterns of tolerance to physiological stress have consequence on  
446 telomere attrition, and possibly on tissue functioning as suggested by Carneiro et al. (2016).

447 Mitochondria are widely recognized as a source of ROS in animal cells, where it is assumed  
448 that overproduction of ROS may conduct to an overwhelmed antioxidant system and  
449 oxidative stress (Quijano et al. 2016). Therefore, an elevated mitochondrial metabolism could  
450 increase the production of ROS and would therefore require an activation of anti-oxidant  
451 defences to maintain the oxidative balance. In this study, the expression of *cat*, *sod1* or *sod2*  
452 genes, encoding enzymes involved in the main mitochondrial anti-oxidant defences, were not  
453 significantly modified after 2 weeks in freshwater in the gills of the tolerant fish compared to  
454 seawater controls. These results are consistent with Ghanavatinasab et al. (2019), where no  
455 significant difference in SOD and CAT were observed in yellowfin seabream *Acanthopagrus*  
456 *sheim* exposed for 2 weeks in 5 ppt water. However, a significant decrease in *cat* expression  
457 levels was measured in the gills of FW-intolerant sea bass compared to FW-tolerant and SW.  
458 This result suggests that telomere attrition in FW-intolerant fish could be due to an imbalance

459 between increased ROS production and downregulated antioxidant defences, leading to  
460 oxidative damage on telomeres in individuals with lower capacity to induce *tert*. But this  
461 hypothesis needs to be further explored by investigating pro-oxidants, other enzymatic and  
462 non-enzymatic anti-oxidant defences as well as oxidative damages.

463 This study suggests that, in case of elevated physiological and metabolic stress, telomere  
464 repair is not prioritised and that energetic limitation has direct consequence on telomere  
465 maintenance. These results are consistent with the hypothesis of Angelier et al (2018)  
466 suggesting a trade-off between immediate protection and telomere maintenance. Additional  
467 evidence concerning energy metabolism, oxidative stress and damage would be necessary to  
468 support the preliminary results of this study. Another recent *in vivo* study highlighted that TL  
469 and metabolism are more tightly linked than initially thought (Casagrande and Hau, 2019).  
470 The results obtained in this study are in agreement with the *metabolic telomere attrition*  
471 *concept* proposed by Casagrande and Hau (2019), that assumes that TL attrition is strongest  
472 during times of energy limitation. Oxidative stress may also be at stake but the relationship  
473 between ROS production and mitochondrial energy production remains to be further  
474 investigated (Salin et al., 2015). In marine teleost, there is no evidence that hyposaline stress  
475 triggers oxidative stress as shown in hepatic tissue of *D. labrax* (Sinha et al. 2015) as well as  
476 in *A. sheim* gills (Ghanavatinasab et al. 2019). But again, the gill was poorly studied and a  
477 transient increase of production of metabolic ROS, as a by-product of cellular respiration  
478 cannot be excluded. According to these hypotheses, telomere dynamics can be considered as a  
479 major determinant for cell homeostasis.

480 Finally, our results suggested that, in the wild, freshwater environment requiring active ionic  
481 regulation would potentially not represent a stress involving telomere shortening in fish  
482 having large salinity tolerance capacity, if salinity variation is considered solely. But in  
483 transitional waters, other environmental parameters are at stake. In particular, temperature and

484 hypoxia have been shown to upregulate TERT expression in testis and liver of medaka and  
485 decrease TL in muscle and fin in brown trout, respectively (Yu et al. 2006; Debes et al. 2016).  
486 Multi-stress experimental studies would be necessary to further understand the influence of  
487 abiotic factors on telomere length, but the results obtained from this study bring interesting  
488 information regarding the consequences of exposure to harsh low salinity conditions and the  
489 intra-specific variation in telomere dynamics on non-model fish vertebrates, which is of  
490 particular interest for ecologists in the context of global change. Therefore, an interesting  
491 perspective of this work would be to determine whether marked fluctuations of environmental  
492 parameters, such as those encountered in transitional waters, affect TL in the wild, in  
493 association with other life-history traits markers such as otolithometry in order to gain further  
494 information on age, growth rate and habitat (Darnaude and Hunter 2017; Bouchoucha et al.  
495 2018).

## 496 **Conclusion**

497 The q-PCR method performed in this study was efficient to detect relative telomere length  
498 changes in sea bass exposed to freshwater. Differences in telomere dynamics in the gills was  
499 linked with individual phenotypic plasticity related to freshwater tolerance. Lower telomere  
500 dynamics (telomere length and *tert* expression) in FW-I was correlated with a higher aerobic  
501 metabolism as well as a lower antioxidant defences.

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## 512 **Ethics**

513 The experiments were conducted according to the guidelines of the European Union (directive  
514 86/609) and of the French law (decree 87/848) regulating animal experimentation. The  
515 experimental design has been approved by the French legal requirement concerning welfare  
516 of experimental animals (APAFIS permit no. 9045-201701068219555).

## 517 **References**

- 518 Ahmed W, Lingner J (2017) Impact of oxidative stress on telomere biology. *Differentiation*  
519 99:. doi: 10.1016/j.diff.2017.12.002
- 520 Almeida-Val VMF, Oliveira AR, da Silva M de NP, et al (2011) Anoxia- and hypoxia-  
521 induced expression of LDH-A\* in the Amazon Oscar, *Astronotus crassipinis*. *Genet Mol*  
522 *Biol* 34:315–322. doi: 10.1590/S1415-47572011000200025
- 523 Anchelin M, Alcaraz-Perez F, Martinez CM, et al (2013) Premature aging in telomerase-  
524 deficient zebrafish. *Dis Model Mech* 6:1101–1112. doi: 10.1242/dmm.011635
- 525 Angelier F, Costantini D, Blévin P, Chastel O (2018) Do glucocorticoids mediate the link  
526 between environmental conditions and telomere dynamics in wild vertebrates? A review.  
527 *Gen Comp Endocrinol* 256:99–111. doi: 10.1016/j.ygcen.2017.07.007
- 528 Appleby SK (2016) Telomere length as a putative biomarker of health and disease.
- 529 Aubert G, Lansdorp PM (2008) Telomeres and aging. *Physiol Rev* 88:557–579. doi:



- 530 10.1152/physrev.00026.2007
- 531 Bertollo L, Cioffi M, Moreira-Filho O (2015) Direct chromosome preparation from  
532 freshwater teleost fishes. In: Fish Cytogenetic Techniques. pp 21–26
- 533 Blackburn EH (1991) Structure and function of telomeres. *Nature* 350:569–573. doi:  
534 10.1038/350569a0
- 535 Blackburn EH (2005) Telomeres and telomerase: their mechanisms of action and the effects  
536 of altering their functions. *FEBS Lett* 579:859–862. doi: 10.1016/j.febslet.2004.11.036
- 537 Blackburn EH, Gall JG (1978) A tandemly repeated sequence at the termini of the  
538 extrachromosomal ribosomal RNA genes in *Tetrahymena*. *J Mol Biol* 120:33–53. doi:  
539 10.1016/0022-2836(78)90294-2
- 540 Boonekamp JJ, Bauch C, Mulder E, Verhulst S (2017) Does oxidative stress shorten  
541 telomeres? *Biol Lett* 13:. doi: 10.1098/rsbl.2017.0164
- 542 Bouchoucha M, Pécheyran C, Gonzalez JL, et al (2018) Otolith fingerprints as natural tags to  
543 identify juvenile fish life in ports. *Estuar Coast Shelf Sci* 212:. doi:  
544 10.1016/j.ecss.2018.07.008
- 545 Burton T, Metcalfe NB (2014) Can environmental conditions experienced in early life  
546 influence future generations? *Proc R Soc B Biol Sci* 281:. doi: 10.1098/rspb.2014.0311
- 547 Carneiro MC, Henriques CM, Nabais J, et al (2016) Short telomeres in key tissues initiate  
548 local and systemic aging in zebrafish. *PLoS Genet* 12:1–31. doi:  
549 10.1371/journal.pgen.1005798
- 550 Casagrande S, Hau M (2019) Telomere attrition: Metabolic regulation and signalling  
551 function? *Biol Lett* 15:. doi: 10.1098/rsbl.2018.0885

- 552 Cawthon RM (2009) Telomere length measurement by a novel monochrome multiplex  
553 quantitative PCR method. *Nucleic Acids Res* 37:e21–e21. doi: 10.1093/nar/gkn1027
- 554 Cawthon RM (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Res* 30:e47–  
555 e47. doi: 10.1093/nar/30.10.e47
- 556 Chang C-H, Mayer M, Rivera-Ingraham G, et al (2021) Effects of temperature and salinity on  
557 antioxidant responses in livers of temperate (*Dicentrarchus labrax*) and tropical (*Chanos*  
558 *Chanos*) marine euryhaline fish. *J Therm Biol* 99:103016. doi:  
559 <https://doi.org/10.1016/j.jtherbio.2021.103016>
- 560 Chang JC-H, Wu S-M, Tseng Y-C, et al (2007) Regulation of glycogen metabolism in gills  
561 and liver of the euryhaline tilapia (*Oreochromis mossambicus*) during acclimation to  
562 seawater. *J Exp Biol* 210:3494–3504. doi: 10.1242/jeb.007146
- 563 Chatelain M, Drobniak SM, Szulkin M (2020) The association between stressors and  
564 telomeres in non-human vertebrates: a meta-analysis. *Ecol Lett* 23:381–398. doi:  
565 10.1111/ele.13426
- 566 Claireaux G, Lagardère J-P (1999) Influence of temperature, oxygen and salinity on the  
567 metabolism of the European sea bass. *J Sea Res* 42:157–168. doi: 10.1016/S1385-  
568 1101(99)00019-2
- 569 Dagarag M, Evazyan T, Rao N, Effros RB (2004) Genetic manipulation of telomerase in  
570 HIV-specific CD8+ T cells: enhanced antiviral functions accompany the increased  
571 proliferative potential and telomere length stabilization. *J Immunol* 173:6303–6311. doi:  
572 10.4049/jimmunol.173.10.6303
- 573 Darnaude A, Hunter E (2017) Validation of otolith  $\delta^{18}\text{O}$  values as effective natural tags for  
574 shelf-scale geolocation of migrating fish. *Mar Ecol Prog Ser AdvView*: doi:

575 10.3354/meps12302

576 Debes P V., Visse M, Panda B, et al (2016) Is telomere length a molecular marker of past  
577 thermal stress in wild fish? *Mol Ecol* 25:5412–5424. doi: 10.1111/mec.13856

578 Driedzic WR, MacIntyre AB, McMorrnan LE (1980) Lactate - The preferred aerobic fuel of  
579 metabolism of the fish heart. In: GILLES RBT-A and EF (ed). Pergamon, pp 67–68

580 Dugdale HL, Richardson DS (2018) Heritability of telomere variation: it is all about the  
581 environment! *Philos Trans R Soc Lond B Biol Sci* 373:20160450. doi:  
582 10.1098/rstb.2016.0450

583 Elcock AH, McCammon JA (1996) Evidence for electrostatic channeling in a fusion protein  
584 of malate dehydrogenase and citrate synthase. *Biochemistry* 35:12652–12658. doi:  
585 10.1021/bi9614747

586 Epel ES, Blackburn EH, Lin J, et al (2004) Accelerated telomere shortening in response to life  
587 stress. *Proc Natl Acad Sci* 101:17312–17315. doi: 10.1073/pnas.0407162101

588 Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill: dominant site of gas  
589 exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste.  
590 *Physiol Rev* 85:97–177. doi: 10.1152/physrev.00050.2003

591 Foote CG, Vleck D, Vleck CM (2013) Extent and variability of interstitial telomeric  
592 sequences and their effects on estimates of telomere length. *Mol Ecol Resour* 13:417–  
593 428. doi: 10.1111/1755-0998.12079

594 Ghanavatinasab Y, Salati AP, Movahedinia A, Shahriari A (2019) Changes in gill antioxidant  
595 status in *Acanthopagrus sheim* exposed to different environmental salinities. *Iran J Sci*  
596 *Technol Trans A Sci* 43:1479–1483. doi: 10.1007/s40995-018-0663-0

- 597 Goldenthal MJ, Marin-Garcia J, Ananthakrishnan R (1998) Cloning and molecular analysis of  
598 the human citrate synthase gene. *Genome* 41:733–738. doi: 10.1139/g98-074
- 599 Hatakeyama H, Nakamura KI, Izumiyama-Shimomura N, et al (2008) The teleost *Oryzias*  
600 *latipes* shows telomere shortening with age despite considerable telomerase activity  
601 throughout life. *Mech Ageing Dev* 129:550–557. doi: 10.1016/j.mad.2008.05.006
- 602 Hausmann M (2010) Telomeres: Linking stress and survival, ecology and evolution. *Curr*  
603 *Zool* 56:. doi: 10.1093/czoolo/56.6.714
- 604 Hausmann MF, Winkler DW, Vleck CM (2005) Longer telomeres associated with higher  
605 survival in birds. *Biol Lett* 1:212–214. doi: 10.1098/rsbl.2005.0301
- 606 Henriques CM, Carneiro MC, Tenente IM, et al (2013) Telomerase is required for zebrafish  
607 lifespan. *PLoS Genet* 9:. doi: 10.1371/journal.pgen.1003214
- 608 Horn T, Gemmell NJ, Robertson BC, Bridges CR (2008) Telomere length change in  
609 European sea bass (*Dicentrarchus labrax*). *Aust J Zool* 56:207–210. doi:  
610 10.1071/ZO08046
- 611 Houben MJM, Moonen HJJ, van Schooten FJ, Hageman GJ (2008) Telomere length  
612 assessment: biomarker of chronic oxidative stress? *Free Radic Biol Med* 44:235–246.  
613 doi: 10.1016/j.freeradbiomed.2007.10.001
- 614 Hwang PP, Lee TH (2007) New insights into fish ion regulation and mitochondrion-rich cells.  
615 *Comp Biochem Physiol - A Mol Integr Physiol* 148:479–497. doi:  
616 10.1016/j.cbpa.2007.06.416
- 617 Joyce W, Ozolina K, Mauduit F, et al (2016) Individual variation in whole-animal hypoxia  
618 tolerance is associated with cardiac hypoxia tolerance in a marine teleost. *Biol Lett* 12:.

619 doi: 10.1098/rsbl.2015.0708

620 Kang CK, Yang WK, Lin ST, et al (2013) The acute and regulatory phases of time-course  
621 changes in gill mitochondrion-rich cells of seawater-acclimated medaka (*Oryzias*  
622 *dancena*) when exposed to hypoosmotic environments. *Comp Biochem Physiol - A Mol*  
623 *Integr Physiol* 164:181–191. doi: 10.1016/j.cbpa.2012.08.010

624 Kurz DJ, Decary S, Hong Y, et al (2004) Chronic oxidative stress compromises telomere  
625 integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci*  
626 117:2417–2426. doi: 10.1242/jcs.01097

627 L'Honoré T, Farcy E, Blondeau-Bidet E, Lorin-Nebel C (2020) Inter-individual variability in  
628 freshwater tolerance is related to transcript level differences in gill and posterior kidney  
629 of European sea bass. *Gene* 741:144547. doi: <https://doi.org/10.1016/j.gene.2020.144547>

630 L'Honoré T, Farcy E, Chatain B, et al (2019) Are European sea bass as euryhaline as  
631 expected? Intraspecific variation in freshwater tolerance. *Mar Biol* 166:102. doi:  
632 10.1007/s00227-019-3551-z

633 Lai T-P, Wright WE, Shay JW (2018) Comparison of telomere length measurement methods.  
634 *Philos Trans R Soc B Biol Sci* 373:20160451. doi: 10.1098/rstb.2016.0451

635 Lambert AJ, Brand MD (2009) Reactive oxygen species production by mitochondria.  
636 *Methods Mol Biol* 554:165–181. doi: 10.1007/978-1-59745-521-3\_11

637 López de Abechuco E, Bilbao E, Soto M, Díez G (2014) Molecular cloning and measurement  
638 of telomerase reverse transcriptase (TERT) transcription patterns in tissues of European  
639 hake (*Merluccius merluccius*) and Atlantic cod (*Gadus morhua*) during aging. *Gene*  
640 541:8–18. doi: 10.1016/j.gene.2014.03.006

- 641 Lorin-Nebel C, Boulo V, Bodinier C, Charmantier G (2006) The Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> cotransporter in  
642 the sea bass *Dicentrarchus labrax* during ontogeny: involvement in osmoregulation. *J*  
643 *Exp Biol* 209:4908–4922. doi: 10.1242/jeb.02591
- 644 Masroor W, Farcy E, Gros R, Lorin-Nebel C (2018) Effect of combined stress (salinity and  
645 temperature) in European sea bass *Dicentrarchus labrax* osmoregulatory processes.  
646 *Comp Biochem Physiol -Part A Mol Integr Physiol* 215:45–54. doi:  
647 10.1016/j.cbpa.2017.10.019
- 648 Mathur MB, Epel E, Kind S, et al (2016) Perceived stress and telomere length: A systematic  
649 review, meta-analysis, and methodologic considerations for advancing the field. *Brain*  
650 *Behav Immun* 54:158–169. doi: 10.1016/j.bbi.2016.02.002
- 651 McLennan D, Armstrong JD, Stewart DC, et al (2018) Telomere elongation during early  
652 development is independent of environmental temperatures in Atlantic salmon. *J Exp*  
653 *Biol* 221:. doi: 10.1242/jeb.178616
- 654 Mierzwa AS, Nguyen F, Xue M, Jonz MG (2020) Regeneration of the gill filaments and  
655 replacement of serotonergic neuroepithelial cells in adult zebrafish (*Danio rerio*). *Respir*  
656 *Physiol Neurobiol* 274:103366. doi: <https://doi.org/10.1016/j.resp.2019.103366>
- 657 Mitter K, Kotoulas G, Magoulas A, et al (2009) Evaluation of candidate reference genes for  
658 QPCR during ontogenesis and of immune-relevant tissues of European sea bass  
659 (*Dicentrarchus labrax*). *Comp Biochem Physiol - B Biochem Mol Biol* 153:340–347.  
660 doi: 10.1016/j.cbpb.2009.04.009
- 661 Monaghan P (2014) Organismal stress, telomeres and life histories. *J Exp Biol* 217:57–66.  
662 doi: 10.1242/jeb.090043
- 663 Monaghan P, Haussmann M (2006) Do telomere dynamics link lifestyle and lifespan? *Trends*

- 664 Ecol Evol 21:47–53. doi: 10.1016/j.tree.2005.11.007
- 665 Naslund J, Pauliny A, Blomqvist D, Johnsson JI (2015) Telomere dynamics in wild brown  
666 trout: effects of compensatory growth and early growth investment. *Oecologia*  
667 177:1221–1230. doi: 10.1007/s00442-015-3263-0
- 668 Nebel C, Romestand B, Nègre-Sadargues G, et al (2005) Differential freshwater adaptation in  
669 juvenile sea-bass *Dicentrarchus labrax*: involvement of gills and urinary system. *J Exp*  
670 *Biol* 208:3859 LP – 3871
- 671 Nilsson GE (2007) Gill remodeling in fish - a new fashion or an ancient secret? *J Exp Biol*  
672 210:2403–2409. doi: 10.1242/jeb.000281
- 673 Nilsson GE, Dymowska A, Stecyk JAW (2012) New insights into the plasticity of gill  
674 structure. *Respir Physiol Neurobiol* 184:214–222. doi: 10.1016/j.resp.2012.07.012
- 675 Olsson M, Wapstra E, Friesen CR (2017) Evolutionary ecology of telomeres: a review. *Ann N*  
676 *Y Acad Sci* 1422:5–28. doi: 10.1111/nyas.13443
- 677 Olsson M, Zhivotovsky B (2011) Caspases and cancer. *Cell Death Differ* 18:1441–1449. doi:  
678 10.1038/cdd.2011.30
- 679 Ozolina K, Shiels HA, Ollivier H, Claireaux G (2016) Intraspecific individual variation of  
680 temperature tolerance associated with oxygen demand in the European sea bass  
681 (*Dicentrarchus labrax*). *Conserv Physiol* 4:1–10. doi: 10.1093/conphys/cov060
- 682 Ozouf-Costaz C, Coutanceau JP, Bonillo C, et al (2015) First insights into karyotype  
683 evolution within the family Mormyridae. *Cybium* 39:227–236
- 684 Paiola M, Knigge T, Duflot A, et al (2018) Oestrogen, an evolutionary conserved regulator of  
685 T cell differentiation and immune tolerance in jawed vertebrates? *Dev Comp Immunol*

- 686 84:48–61. doi: 10.1016/j.dci.2018.01.013
- 687 Pauliny A, Wagner RH, Augustin J, et al (2006) Age-independent telomere length predicts  
688 fitness in two bird species. *Mol Ecol* 15:1681–1687. doi: 10.1111/j.1365-  
689 294X.2006.02862.x
- 690 Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-  
691 PCR. *Nucleic Acids Res* 29:e45–e45
- 692 Polakof S, Arjona FJ, Sangiao-Alvarellos S, et al (2006) Food deprivation alters  
693 osmoregulatory and metabolic responses to salinity acclimation in gilthead sea bream  
694 *Sparus auratus*. *J Comp Physiol B* 176:441–452. doi: 10.1007/s00360-006-0065-z
- 695 Quijano C, Trujillo M, Castro L, Trostchansky A (2016) Interplay between oxidant species  
696 and energy metabolism. *Redox Biol* 8:28–42. doi: 10.1016/j.redox.2015.11.010
- 697 Roche TE, Reed LJ (1974) Monovalent cation requirement for ADP inhibition of pyruvate  
698 dehydrogenase kinase. *Biochem Biophys Res Commun* 59:1341–1348. doi:  
699 10.1016/0006-291x(74)90461-6
- 700 Rogdakis Y, Ramfos A, Koukou K, et al (2010) Feeding habits and trophic level of sea bass  
701 (*Dicentrarchus labrax*) in the Messolonghi-Etoliko lagoons complex (Western Greece). *J*  
702 *Biol Res* 13:13–26
- 703 Rollings N, Miller E, Olsson M (2014) Telomeric attrition with age and temperature in  
704 Eastern mosquitofish (*Gambusia holbrooki*). *Naturwissenschaften* 101:241–244. doi:  
705 10.1007/s00114-014-1142-x
- 706 Sadoul B, Alfonso S, Bessa E, et al (2018) Enhanced brain expression of genes related to cell  
707 proliferation and neural differentiation is associated with cortisol receptor expression in



- 708 fishes. *Gen Comp Endocrinol* 267:76–81. doi: 10.1016/j.ygcen.2018.06.001
- 709 Sales CF, Santos KPE dos, Rizzo E, et al (2017) Proliferation, survival and cell death in fish  
710 gills remodeling: From injury to recovery. *Fish Shellfish Immunol* 68:10–18. doi:  
711 <https://doi.org/10.1016/j.fsi.2017.07.001>
- 712 Saretzki G (2018) Telomeres, Telomerase and Ageing BT - *Biochemistry and Cell Biology*  
713 of Ageing: Part I Biomedical Science. In: Harris JR, Korolchuk VI (eds). Springer  
714 Singapore, Singapore, pp 221–308
- 715 Scott GR, Schulte PM (2005) Intraspecific variation in gene expression after seawater transfer  
716 in gills of the euryhaline killifish *Fundulus heteroclitus*. *Comp Biochem Physiol - A Mol*  
717 *Integr Physiol* 141:176–182. doi: 10.1016/j.cbpb.2005.05.002
- 718 Simide R, Angelier F, Gaillard S, Stier A (2016) Age and heat stress as determinants of  
719 telomere length in a long-lived fish, the siberian sturgeon. *Physiol Biochem Zool*  
720 89:441–447. doi: 10.1086/687378
- 721 Sinha AK, Abdelgawad H, Zinta G, et al (2015) Nutritional status as the key modulator of  
722 antioxidant responses induced by high environmental ammonia and salinity stress in  
723 European sea bass (*Dicentrarchus labrax*). *PLoS One* 10:e0135091
- 724 Smith EM, Pendlebury DF, Nandakumar J (2020) Structural biology of telomeres and  
725 telomerase. *Cell Mol Life Sci* 77:61–79. doi: 10.1007/s00018-019-03369-x
- 726 Sola L, Bressanello S, Rossi AR, et al (1993) A karyotype analysis of the genus *Dicentrarchus*  
727 by different staining techniques. *J Fish Biol* 43:329–337. doi: doi:10.1111/j.1095-  
728 8649.1993.tb00567.x
- 729 Sollid J (2005) Temperature alters the respiratory surface area of crucian carp *Carassius*

- 730 *carassius* and goldfish *Carassius auratus*. J Exp Biol 208:1109–1116. doi:  
731 10.1242/jeb.01505
- 732 Sollid J, Nilsson GE (2006) Plasticity of respiratory structures - Adaptive remodeling of fish  
733 gills induced by ambient oxygen and temperature. Respir Physiol Neurobiol 154:241–  
734 251. doi: 10.1016/j.resp.2006.02.006
- 735 Starkweather AR, Alhaeeri AA, Montpetit A, et al (2014) An integrative review of factors  
736 associated with telomere length and implications for biobehavioral research. Nurs Res  
737 63:36–50. doi: 10.1097/NNR.0000000000000009
- 738 Toupance S, Villemonais D, Germain D, et al (2019) The individual's signature of telomere  
739 length distribution. Sci Rep 9:685. doi: 10.1038/s41598-018-36756-8
- 740 Tseng Y-C, Lee J-R, Chia J, et al (2008) Regulation of lactate dehydrogenase in tilapia  
741 (*Oreochromis mossambicus*) gills during acclimation to salinity challenge. Zool Stud  
742 47:473–480
- 743 Tseng YC, Hwang PP (2008) Some insights into energy metabolism for osmoregulation in  
744 fish. Comp Biochem Physiol - C Toxicol Pharmacol 148:419–429. doi:  
745 10.1016/j.cbpc.2008.04.009
- 746 Tzaneva V, Vadeboncoeur C, Ting J, Perry SF (2014) Effects of hypoxia-induced gill  
747 remodelling on the innervation and distribution of ionocytes in the gill of goldfish,  
748 *Carassius auratus*. J Comp Neurol 522:118–130. doi: 10.1002/cne.23392
- 749 Valvona CJ, Fillmore HL, Nunn PB, Pilkington GJ (2016) The regulation and function of  
750 lactate dehydrogenase A: therapeutic potential in brain tumor. Brain Pathol 26:3–17. doi:  
751 10.1111/bpa.12299

- 752 Vandesompele J, De Preter K, Pattyn F, et al (2002) Accurate normalization of real-time  
753 quantitative RT-PCR data by geometric averaging of multiple internal control genes.  
754 Genome Biol 3:research0034.1. doi: 10.1186/gb-2002-3-7-research0034
- 755 Vijayan M, Morgan J, Sakamoto T, et al (1996) Food-deprivation affects seawater  
756 acclimation in tilapia: hormonal and metabolic changes. J Exp Biol 199:2467 LP – 2475
- 757 Wang F, Pan X, Kalmbach K, et al (2013) Robust measurement of telomere length in single  
758 cells. Proc Natl Acad Sci U S A 110:. doi: 10.1073/pnas.1306639110
- 759 Wilbourn R V., Moatt JP, Froy H, et al (2018) The relationship between telomere length and  
760 mortality risk in non-model vertebrate systems: A meta-analysis. Philos Trans R Soc B  
761 Biol Sci 373:. doi: 10.1098/rstb.2016.0447
- 762 Yu RMK, Chen EXH, Kong RYC, et al (2006) Hypoxia induces telomerase reverse  
763 transcriptase (TERT) gene expression in non-tumor fish tissues in vivo: The marine  
764 medaka (*Oryzias melastigma*) model. BMC Mol Biol 7:1–12. doi: 10.1186/1471-2199-7-  
765 27
- 766

**Figure 1** Fluorescent in situ hybridisation (FISH) of metaphase chromosomes isolated from head-kidneys of 10 month-old European sea bass using the telomeric probe (TTAGGG)<sub>7</sub> labelled with Cy3 at its 5' end, indicated by red colour. 2 males and 2 females were analysed with N = 3 slides per fish. Scale bar: 10µm

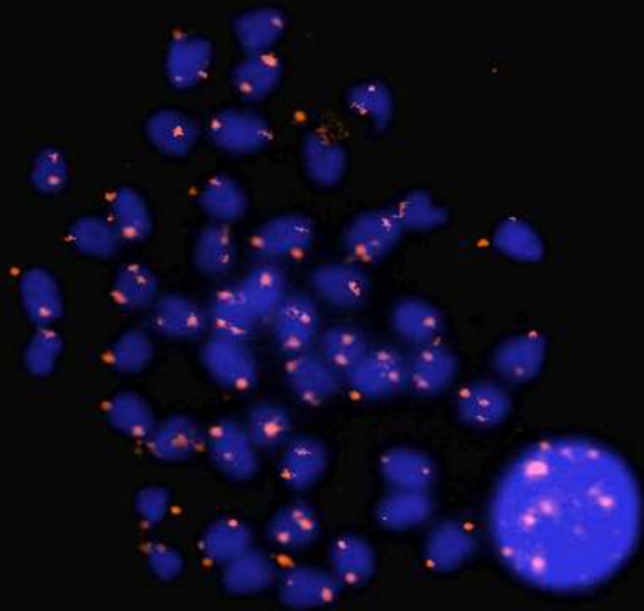
**Figure 2** Relative telomere length expressed as T/R ratio calculated using  $\Delta\Delta\text{Ct}$  method normalised against the single copy gene *mc2r* in gills of 5 month-old sea bass maintained in seawater and after a transfer of 2 weeks in fresh water. Different letters denote significant differences between groups (Mann-Whitney test, Bonferroni-corrected  $P < 0.0167$ , means  $\pm$  s.e.m, N=10-14). SW: control fish in seawater, FW-T: FW-tolerant fish, FW-I: FW-intolerant fish

**Figure 3** Relative mRNA expression of genes involved in telomere maintenance (a), cell proliferation (b) and apoptosis (c, d) in the gill of 5 month-old sea bass maintained in seawater (SW) or exposed for 2 weeks to fresh water (FW-T and FW-I). (a) telomerase catalytic subunit *tert* (b) proliferation cell nuclear antigen *pcna* (c) caspase 8 *casp8* (d) caspase 9 *casp9*. The mRNA expression was calculated using the  $\Delta\Delta\text{Ct}$  method with SW as a reference and normalised according to the expression of two reference genes *113* and *18S*. Different letters denote significant differences between phenotypes (Mann-Whitney test, Bonferroni-corrected  $P < 0.0167$ , means  $\pm$  s.e.m, N=10-15). SW: control fish in seawater, FW-T: FW-tolerant fish, FW-I: FW-intolerant fish

**Figure 4** Relative mRNA expression of genes involved in antioxidant defence (a, b, c) and metabolism (d, e) in the gill of 5 month-old sea bass maintained in seawater (SW) or exposed for 2 weeks to fresh water (FW-T and FW-I). (a) superoxide dismutase 1 *sod1* (b) superoxide dismutase 2 *sod2* (c) catalase *cat* (d) ATP citrate synthase *cs* (e) lactate dehydrogenase a (*ldh-a*). The mRNA expression was calculated using the  $\Delta\Delta\text{Ct}$  method with SW as a reference and normalised according to the expression of two reference genes *113* and *18S*. Different letters denote significant differences between groups (Mann-Whitney test, Bonferroni-corrected  $P < 0.0167$ , means  $\pm$  s.e.m, N=10-15). SW: control fish in seawater, FW-T: FW-tolerant fish, FW-I: FW-intolerant fish

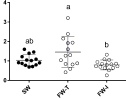
**Table 1** Primer sequences used for relative telomere length and gene expression analysis

**Table 2** Spearman  $r$ -correlation matrix. Asterisks denotes significant  $r$  using  $P < 0.05$ .

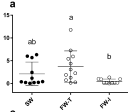




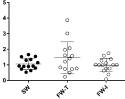
casp8 relative expression



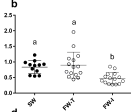
tert relative expression

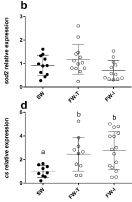
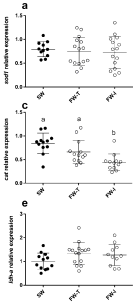


casp8 relative expression



poma relative expression







Target gene	Primer name	Sequences ID	Sequence (from 5' to 3')	Efficiency	Reference
<i>pcna</i>	PCNA F	DLAgn_00120330	CAGAGCGGCTGGTTGCA	1.7	Sadoul et al., 2018
	PCNA R		CACCAAAGTGGAGCGAACAA		
<i>tert</i>	TERT F	DLAgn_00199170	GGGTCAGGGGCTTCTTGAC	2.1	This study
	TERT R		AGAAACAGGCTCGAACCCAGG		
<i>casp8</i>	CASP8 F	FJ225665	TGTCAGGGAAGCCTCTACCA	2.1	Paiola et al., 2018
	CASP8 R		CATCCCAGCAGGAAGTCAG		
<i>casp9</i>	CASP9 F	DQ345775	CGAATGCAACCGAGCACAAA	1.9	Paiola et al., 2018
	CASP9 R		ACTAACGACCGCCAATGAGG		
<i>tel</i>	TEL G		ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT	2	Cawthon et al., 2009
	TEL C		TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA		
<i>l13</i>	L13 F	DT044539	TCTGGAGGACTGTCAGGGGCATGC	2	Mitter et al., 2009
	L13 R		AGACGCACAATCTTGAGAGCAG		
<i>mc2r</i>	MC2R F	FR870225	CATCTACGCCTTCCGCATTG	2	Samaras & Pavlidis, 2018

	MC2R R		ATGAGCACCGCCTCCATT		
<i>18s</i>	18S F	KU820862	AGGAATTGACGGAAGGGCAC	2	Masroor et al., 2018
	18S R		TAAGAACGGCCATGCACCAC		
<i>sod1</i>	SOD1 F	DLA_LG14_005480	AACCATGGTGATCCACGAGA	1.9	Chang et al, 2021
	SOD1 R		ATGCCGATGACTCCACAGG		
<i>sod2</i>	SOD2 F	DLAgn_00071530	TGCCCTCCAGCCTGCTCT	1.7	Chang et al, 2021
	SOD2 R		CTTCTGGAAGGAGCCAAAGTC		
<i>cat</i>	CAT F	DLAgn_00171080	TGCTGAATGAAGAGGAGCGC	2	This study
	CAT R		ACAGCCTTCAAGTTCTGCAAC		
<i>cs</i>	CS F	DLAgn_00102430	TGGCGTCTATGAAAGTGTGG	1.9	This study
	CS R		CTGAAGTGAACATGGTGGCG		
<i>ldh-a</i>	LDHA F	DLAgn_00166080	TGACGCTGAGAACTGGAAGG	2	This study
	LDHA R		GTGCAGGTTCTTGAGGATGC		

	Body length (cm)	Body mass (g)	TL ( <i>tel/mc2r</i> )	<i>tert</i>	<i>pcna</i>	<i>Casp8</i>	<i>casp9</i>	<i>sod1</i>	<i>sod2</i>	<i>cat</i>	<i>cs</i>	<i>ldh-a</i>
Body length (cm)	1,00											
Body mass (g)	0,96*	1,00										
TL ( <i>tel/mc2r</i> )	0,26	0,18	1,00									
<i>tert</i>	0,16	0,18	0,48*	1,00								
<i>pcna</i>	0,23	0,31*	0,48*	0,31	1,00							
<i>casp8</i>	0,06	0,10	0,38*	0,64*	0,48*	1,00						
<i>casp9</i>	0,01	0,00	0,21	0,47*	0,28	0,78*	1,00					
<i>sod1</i>	0,00	0,15	-0,28	-0,05	0,52*	0,15	0,14	1,00				
<i>sod2</i>	0,24	0,23	0,18	0,66*	0,31	0,60*	0,57*	0,15	1,00			
<i>cat</i>	0,49*	0,44*	0,59*	0,26	0,28	0,18	0,06	-0,17	0,24	1,00		
<i>cs</i>	-0,31	-0,22	-0,58*	0,11	-0,09	0,45*	0,52*	0,12	0,34	-0,66*	1,00	
<i>ldh-a</i>	-0,30	-0,21	-0,39*	-0,12	-0,38*	-0,04	-0,13	0,02	-0,15	-0,26	0,48*	1,00