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## Temporal variations in scale cortisol indicate consistent local-and broad-scale constraints in a wild marine teleost fish

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### Abstract :

Environmental changes can alter the nursery function of coastal areas through their impact on juveniles' growth and survival rates, an effect mediated by individuals' chronic stress response. Fish chronic stress can be quantified using scale cortisol but no study has yet been quantified the spatio-temporal variations in scale cortisol and its relationship with growth in wild nurseries. We collected wild sea bass juveniles (*Dicentrarchus labrax*, four years, three nurseries) and found that scale cortisol levels increased consistently with age and across cohorts in 2019 and 2020 probably due to greater stress history in older fish and/or heatwaves that occurred in summers of 2018 and 2019. Growth was impaired in fish with high scale cortisol in 2019 and 2020, confirming the usefulness of scale cortisol as a biomarker of broad and local constraints in wild fish; longer time series will enable us to identify environmental factors underpinning these temporal variations.

### Highlights

► Environment's effect on individuals' growth is mediated by their chronic stress ► We measured scale cortisol in juvenile sea bass aged 1–4+ during 2017–2020 ► Scale cortisol increased substantially in 2019 and 2020 in all nurseries/age ► High scale cortisol was related to lower growth only in 2019 and 2020 ► Scale cortisol might reflect local to broad-scale environmental constraints

**Keywords :** Allostatic load, Chronic stress, Fitness, Fish, Growth, Nurseries

## 40 1. INTRODUCTION

41 Global climate changes and ever-increasing anthropogenic activities threaten the functioning  
42 of key habitats such as nurseries. Indeed, coastal and estuarine areas often have high  
43 concentrations of juveniles of many marine species that feed on their rich benthic communities  
44 hereby sustaining juvenile growth (Beck et al., 2001). Nurseries' function, defined as their  
45 contribution to the recruitment of new individuals to adult populations (Beck et al., 2001),  
46 therefore depends on juveniles' density and their individuals' growth and survival rates. The  
47 expression of these two interdependent life history traits (e.g. in fish; Crossin, Cooke,  
48 Goldbogen, & Phillips, 2014; Gislason et al., 2010) is optimal within a range of environmental  
49 conditions (Stearns, 1992), and deviations from this range are perceived by individuals as  
50 threats to their homeostasis (i.e. equilibrium state) triggering a stress response (Greenberg et  
51 al., 2002; McEwen & Wingfield, 2003). While acute stress responses to short perceived  
52 challenges enable individuals to cope with environmental perturbations (Bonier et al., 2009),  
53 chronic stress responses to long-lasting stress factors can have detrimental effects on  
54 individuals' life history traits. Indeed, chronic stress affects multiple physiological processes  
55 and the energy allocated to the stress response can lead to reduced growth and reproductive  
56 investment and ultimately individuals' death (Barton, 2002; Bonier et al., 2009). Therefore,  
57 individuals' stress response can increase their fitness in the short-term but will become  
58 detrimental if sustained; as such intermediate stress responses (due to intermediate  
59 perception or intermediate reactivity) are expected to be optimal (Greenberg et al., 2002).  
60 Consequently, the increasing variation in environmental factors exposes juvenile fish to more  
61 and more frequent stressful environmental conditions that may affect their life history traits and  
62 hence alter nurseries' functioning.

63  
64 In fisheries science, physiological markers have primarily been used to reduce bycatch  
65 mortality (Farrell et al., 2001; Skomal, 2007) but their broader use as indicators of changes in  
66 stocks' states is only becoming more widely recognised (Brosset et al., 2021). Individual stress

67 levels can be measured with a wide variety of biomarkers (Barton et al., 2002), generally based  
68 on glucocorticoids produced by the hypothalamic-pituitary-interrenal (HPI) axis (Mommsen et  
69 al., 1999). Cortisol, the dominant glucocorticoid in teleost fish, is a steroid secreted by  
70 steroidogenic cells located in the interrenal glands in response to stress factors (Barton et al.,  
71 2002; Das et al., 2018; Faught & Vijayan, 2016) but also under normal conditions (e.g.  
72 circadian rhythmicity). When released, cortisol induces changes in multiple physiological  
73 processes (e.g. metabolism, immunity, osmoregulation) and growth (Faught & Vijayan, 2016;  
74 Mommsen et al., 1999) either by regulating cell functions (Das et al., 2018) or by binding to  
75 glucocorticoid or mineralocorticoid receptors (Prunet et al., 2006). In particular, the primary  
76 action of cortisol is considered adaptive as it induces the release of glucose in the blood during  
77 acute stress response, but in case of chronic stress, it will have detrimental effects on for  
78 instance growth (Mommsen et al., 1999). Cortisol, released in the blood, is incorporated in  
79 growing scales through the capillaries vascularising the loose dermis separating scale pockets  
80 (Carbajal et al., 2019; Sire et al., 1997) and its measurement reflects individuals' overall stress  
81 history (Aerts et al., 2015; Laberge et al., 2019). Therefore, scale cortisol is probably the best  
82 suited parameter/tissue combination to investigate chronic stress, as this method provides a  
83 retrospective view on HPI axis (re)activity, enabling to quantify and understand long-term  
84 constraints exerted by the environment on individuals (Aerts et al., 2015; Hanke et al., 2019;  
85 Sadoul & Geffroy, 2019; Weirup et al., 2021). It has been demonstrated that scale cortisol is  
86 independent of acute stress responses and integrates the (re)activity of the HPI axis  
87 retrospectively over time (Aerts et al., 2015; Carbajal et al., 2019; Laberge et al., 2019;  
88 Samaras et al., 2021). Consequently, scale cortisol has been used to quantify fish welfare (e.g.  
89 Goikoetxea et al., 2021; Hanke et al., 2019, Weirup et al., 2021), and only recently have been  
90 used in the wild to study hierarchies in social fish (Culbert et al., 2021) or lake pollution  
91 (Carbajal et al., 2019). As the use of scale cortisol in the wild has yet been limited in scope  
92 (single years, few sites, limited sampling), there is a clear need for larger scale studies  
93 quantifying the effect of environmental variations on fish scale cortisol levels across multiple

94 ages and sites to determine its applicability as indicator of environmental constraints on wild  
95 teleost fish.

96

97 The European sea bass (*Dicentrarchus labrax*, Moronidae) sustains major commercial and  
98 recreative fisheries along western European coasts (Zarauz et al., 2015), but its stocks have  
99 been declining due to the conjunction of high harvest rates and poor recruitments (ICES, 2020).  
100 As sea bass juveniles grow in coastal and estuarine nurseries for *ca.* four years, declines in  
101 recruitment rates can be due to the degradation of their nurseries (Beck et al., 2001; Dahlgren  
102 et al., 2006). Estuaries are particularly favourable areas for the development of sea bass  
103 juveniles as they are rich in prey (e.g. small crabs, shrimps, and fish such as sprat; Aprahamian  
104 & Barr, 1985; Kelley, 1987) and/or hold multiple shelter opportunities favouring predator  
105 avoidance (Courrat et al., 2009). However, estuaries are also complex habitats with highly  
106 variable environments (e.g. oxygenation, salinity, turbidity, temperature, and hydrodynamics;  
107 Levin & Stunz, 2005). Species growing in estuarine nurseries are well-adapted to these  
108 variations (Elliott & Quintino, 2007), but strong anthropogenic pressures (e.g. pollution,  
109 acidification, hypoxia) can impair the growth and survival of sea bass juveniles (e.g. Breitburg  
110 et al., 2018; Vasseur et al., 2014) and might explain the decline in recruitments rates of this  
111 species.

112

113 In this framework, we sampled juveniles of the European sea bass to determine how  
114 environmental constraints impact this species using scale cortisol. Sea bass juveniles (aged 1  
115 to 4 years) were sampled in four consecutive years in three major estuarine nurseries along  
116 France's Western coast (i.e. Gironde, Loire, and Seine estuaries). We first quantified the  
117 spatio-temporal variation in scale cortisol to determine whether scale cortisol levels differed  
118 among nurseries; we had no *a priori* expectation as these estuaries hold wide catchment areas,  
119 many large urban areas, and industries with high historical contamination levels. We also  
120 tested the hypothesis that scale cortisol levels increased with fish age. Two processes  
121 underpin this hypothesis: the accumulation of stressful experiences over time and/or juveniles

122 acclimation to habitats with increasing salinity (mediated by cortisol; McCormick, 2001) as they  
123 move downstream up to age 3-4 (Roy et al., 2022). We then quantified changes in scale  
124 cortisol concentrations within cohorts and nurseries to determine whether there are consistent  
125 changes across nurseries (suggesting broad scale environmental effects) or inconsistent  
126 changes across nurseries (suggesting local environmental effects). We finally tested the hypothesis that  
127 juvenile growth is related to their stress levels, but that only particularly high stress levels have  
128 a negative effect on growth. More specifically, we expected that the effect of cortisol on fish  
129 growth is dependent on individuals' age and sampling year as, for instance, young fish may be  
130 particularly sensitive to high stress levels and years with elevated cortisol levels might be those  
131 in which more deleterious effects of cortisol can be observed.

132

## 133 2. MATERIAL AND METHODS

134

### 135 2.1 Data collection

136 Adult sea bass spawn mainly offshore between mid-January and June. After hatching, larvae  
137 drift towards the coasts and colonise estuaries or sheltered bays where juveniles grow (Le Goff  
138 et al., 2017). At *ca.* age four, sea bass juveniles leave nurseries to reach fattening and breeding  
139 areas. Sea bass juveniles were collected during the NOURDEM survey (Drogou et al., 2019)  
140 which aims at quantifying the abundance of juveniles of demersal fish in Loire (since 2016),  
141 Seine (since 2017), and Gironde (since 2019). The survey takes place every year in early-July  
142 (Loire), early August (Seine), and early-September (Gironde), with dates slightly varying to  
143 minimize tidal currents and changes in upstream salinity limits. In each estuary, *ca.* 70 tows  
144 were performed onboard of local professional trawlers from upstream salinity limits to  
145 estuaries' mouth. These boats were chosen to enable the sampling of foreshore areas at mid-  
146 tides (*ca.* 10 m long, draughts < 2 m; Le Goff et al., 2017). Tows lasted 15 min (+2 to -4 min)  
147 with a traction speed set at 3.5 knots with the Ifremer's 'NOURDEM GOV Trawling net'. This  
148 bottom otter-trawl was specifically designed to capture juveniles of demersal fish of a minimum  
149 size of 3 to 4 cm with its 7 m width, 2.40 m height and 18 mm terminal meshes (Le Goff et al.,

150 2017). We sampled fish throughout each estuary according to the limits of the known size  
151 distributions (ages 1: 15-23 cm, age 2: 23-30 cm, ages 3+: >30 cm) in 2017-2020 (Loire and  
152 Seine) and 2019-2020 (Gironde). After each tow, fish were sorted and sea bass juveniles with  
153 length consistent with target sizes were euthanized and stored frozen at -20 °C until further  
154 treatment in the laboratory (all other sea bass juveniles were released alive, major injuries  
155 being rare; Le Goff et al. 2017). We targeted a maximum of 20 individuals per size class in  
156 each size in each year, but the actual sample size in each age class differed once actual ages  
157 were determined. Authorization and ethical approval for fish sampling provided by national  
158 (DPMA) and regional authorities (Normandie, Pays de la Loire, Nouvelle Aquitaine); National  
159 & regional committees of professional fishermen (CNPMM, CRPM Normandie; COREPMEM  
160 Pays de la Loire, CRPMEM Nouvelle Aquitaine) for 2017-2018 (Ref. 18/2 216 097 AVT1) and  
161 2019-2020 (Ref. Osiris PFEA400018DM0310001; ref. Ifremer: 18/2216441).

162

## 163 **2.2 Laboratory measurements**

164 Once in the laboratory, fish were thawed and individuals' total body length (nearest 0.5 cm),  
165 total uneviscerated weight ( $\pm 0.2$  g), and liver weight ( $\pm 0.01$  g) were measured. Where  
166 possible, we recorded the sex of the fish and maturity stage (through visual inspection), and  
167 we sampled sagittal otoliths to determine fish age based on the number of growth rings. As  
168 there were only a few individuals aged 5 years in the dataset, these fish were combined with  
169 those aged 4 (Suppl. Table 1). Ontogenetic scales were sampled in a standardized manner on  
170 the left flank and dorsal to the lateral line, transported and stored at -20 °C. After removal of  
171 the mucus using Milli-Q water, scales were dried, weighted, and homogenized using  
172 PowerBead tubes (ceramic 2.8 mm, Qiagen) in a bead raptor (PowerLyzer 24, Qiagen; see  
173 Aerts et al. 2015 for more details). Extraction, ultra-purification and subsequent ultra-  
174 performance liquid chromatography coupled to tandem mass spectrometry analysis (UPLC-  
175 MS/MS; Xevo TQS, Waters, Milford, USA) were performed as described in Aerts et al. (2015).  
176 Altogether, scale cortisol was quantified for 721 sea bass juveniles. We conducted a  
177 preliminary test of the method based on ca. 100 fish per site in 2019 and subsequently

178 measured cortisol concentrations for ca. 60 fish per site in 2017, 2018, and 2020 (the number  
179 of fish analysed per age differs in 2019 and 2020 as individuals' age were not known when we  
180 selected the samples to be analysed; Table A.1). On average, scale cortisol levels were  
181 quantified using 61.5 mg of dry scales per fish (sd: 32.7, range: 5.0-215.0) which consisted of  
182 99 scales per fish on average (sd: 78.3, range: 22-493). None of the samples were observed  
183 to have a scale cortisol level below the detection limit ( $CCa = 0.0001 \mu\text{g.kg}^{-1}$ ) of the UPLC-  
184 MS/MS method used (full technical details can be found in Aerts et al. 2015).

185

### 186 **2.3 Data analyses**

187 Scale cortisol concentrations were log-transformed (base 10) to account for the strong right  
188 skew of this variables' distribution (Shapiro-Wilk test,  $W = 0.421$ ,  $P < 0.001$ ,  $N = 721$ ). We  
189 tested whether there were differences in scale cortisol between ages, years, nursery, and the  
190 interactions between age and year using a linear mixed model. The random effect of this model  
191 consisted in a compound variable combining cohorts' identity and nursery's identification code  
192 to account for the non-independence of cortisol levels measured in consecutive ages of the  
193 same cohort within each nursery. The model did not include a three-way interaction between  
194 age, nursery and year as the absence of data from Gironde in 2017 and 2018 led to rank  
195 deficiencies. We calculated the intra-class correlation to quantify the amount of variance  
196 explained by the within nursery correlation in the cortisol levels of the same cohort as:

197

$$\frac{\hat{d}^2}{\hat{d}^2 + \hat{\sigma}^2}$$

198 where  $\hat{d}^2$  is the variance explained by the random intercepts and  $\hat{\sigma}^2$  the residual variance (Zuur  
199 et al., 2007). We estimated parameters using maximum likelihood and compared the relative  
200 performance of the models based on their Akaike Information Criterion for small sample size  
201 (AICc) using the r-package 'MuMIn' 1.43.17 (Barton, 2020). All mixed effect models were  
202 implemented in the r package 'nlme' (Pinheiro et al., 2021).

203



204 We then tested the hypothesis that high scale cortisol levels led to a decline in individuals'  
205 growth using generalised additive models. These models contained individuals' age, sampling  
206 year, and their interaction as cofactors to account for (i) year differences in length and scale  
207 cortisol levels, and (ii) the increase in length and scale cortisol with age (using age as a cofactor  
208 leads to the estimate of the effect of cortisol on individuals' length within each age category,  
209 and hence the effect of cortisol on individuals' growth). The smoothing function of scale cortisol  
210 was therefore estimated separately by age, year, and their interaction in different models. We  
211 set the maximum number of knots to 5 to limit the risks of over-parameterisation. We started  
212 from the most complex model with the interaction Age\*Year as a cofactor and smoothing  
213 specific to each Age\*Year category and simplified the terms of this model. We did not  
214 undertake this analysis in the 2017 age category 4+ as there were only 11 fish in this age-year  
215 class. We then compared the performance of these models using AICs. We did not take into  
216 account differences in the scale cortisol levels among nurseries because of the lack of samples  
217 in specific age-year-site categories (Table A.1). All generalised additive models were  
218 implemented using the r-package 'mgcv' v1.8-31 (Wood et al., 2011, 2016).

219

### 220 3. RESULTS

221

222 Across all observations (N=721), the raw scale cortisol levels in sea bass juveniles ranged  
223 from 0.0008-14.751  $\mu\text{g.kg}^{-1}$  with a median of 0.169  $\mu\text{g.kg}^{-1}$  (IQR: 0.029-0.642  $\mu\text{g.kg}^{-1}$ ). There  
224 was substantial variation in scale cortisol level (Table A.1) which were primarily explained by  
225 individuals' age and sampling year (Table 1). The second best model had a slightly lower  
226 performance than the best model ( $\Delta\text{AIC} = 1.00$ ) and included the sampling nurseries in addition  
227 to individuals' age and sampling year (Table 1). The performance of all other models was lower  
228 ( $\Delta\text{AICc} > 14$ ; Table 1; Table A.2). Therefore, scale cortisol increased with individuals' age, an  
229 effect consistent across nurseries and cohorts (Figure 1, Tables A.1 and A.2). The sampling  
230 year had also a strong influence on scale cortisol levels, with a very large increase between  
231 2018 and 2019, an effect again consistent across ages and cohorts (Figure 1, Tables A.1 and



232 A.2). The difference in scale cortisol between nurseries was clearly weaker than age and year  
 233 differences (Tables A.1 and A.2). Finally, the strong effect of the sampling year on the scale  
 234 cortisol levels led to a low intra-class correlation ( $ICC = 0.046$ ).

235

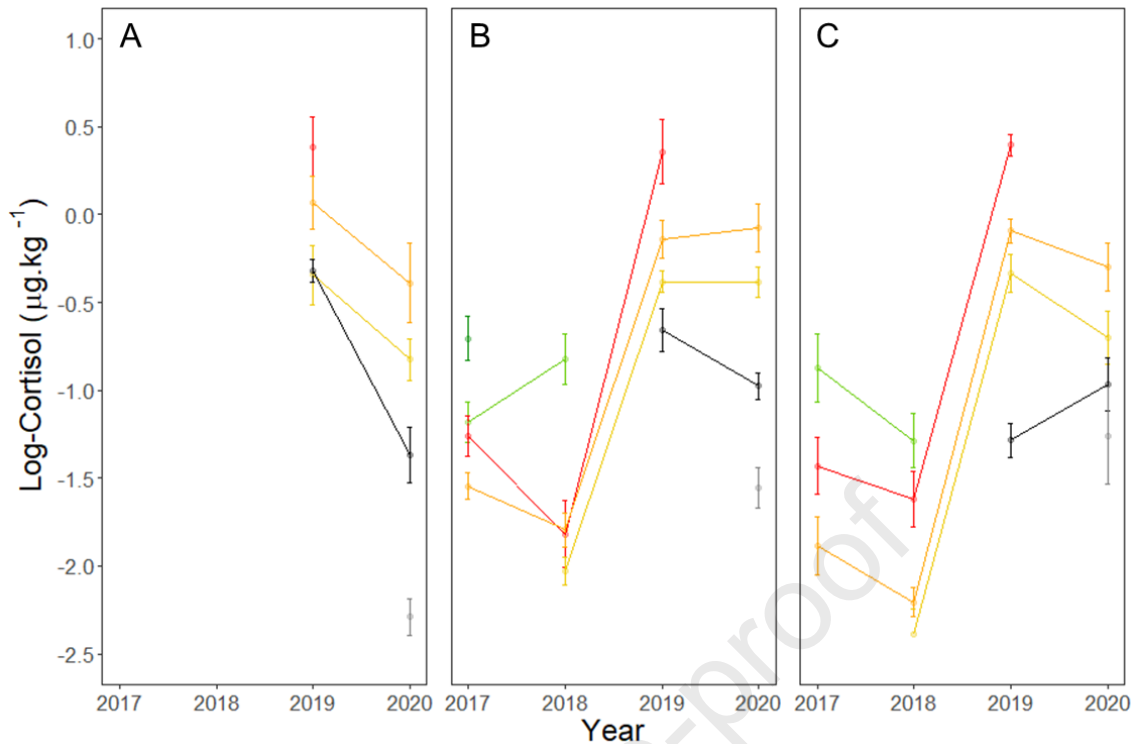
236 Table 1: Relative performance of models testing the effect of individuals' age, sampling year  
 237 and nursery on their scale cortisol levels. Table entries: number of parameters (K), Log-  
 238 likelihood (LogLik), Akaike's Information Criterion for small sample sizes (AICc), difference in  
 239 AICc values relative to the best model ( $\Delta AICc$ ), model weight ( $w_i$ ). Interaction terms are noted  
 240 with ':' between two main effects.

Parameters	K	logLik	AICc	$\Delta AICc$	$w_i$
Age+Year	9	-622.59	1263.40	0.00	0.62
Age+Year+Nursery	11	-621.03	1264.40	1.00	0.38
Age+Year+Nursery+Age:Year	20	-618.30	1277.80	14.36	0.00
Age+Year+Age:Year	18	-620.57	1278.10	14.68	0.00
Year	6	-649.44	1311.00	47.56	0.00
Year+Nursery	8	-648.73	1313.70	50.22	0.00
Age	6	-818.44	1649.00	385.56	0.00
Age+Nursery	8	-817.59	1651.40	387.95	0.00
Nursery	5	-900.22	1810.50	547.09	0.00
Null model	3	-904.36	1814.80	551.31	0.00

241

242

243



244

245 Figure 1. Changes in scale cortisol levels in sea bass juveniles sampled in three estuarine  
 246 nurseries: Gironde (panel A), Loire (panel B), and Seine (panel C). Dots represent the  
 247 averages of scale cortisol concentrations for each age within each nursery and their associated  
 248 standard error. Lines connect the consecutive ages of each cohort that are represented with  
 249 different colours (dark green: 2014, light green: 2015, red: 2016, orange: 2017, yellow: 2018,  
 250 black: 2019, grey: 2020).

251

252 Individuals' scale cortisol concentrations influenced the growth of sea bass juveniles and this  
 253 effect differed substantially among years and ages. Indeed, the two best models contained  
 254 smoothing specific to each age and year category (Table 2) and year and age as cofactors or  
 255 as an interaction for the second best model (Table 2). The adjusted  $R^2$  and deviance explained  
 256 by these two models are similar, but the model without the interaction is more parsimonious  
 257 (Table 2). In this model, the effect of scale cortisol on fish growth differs substantially across  
 258 ages and years (Table A.3), being significant in fish aged 1, 2, 3, and 4+ in 2019, and in fish  
 259 aged 2 and 3 in 2020. Overall, increasing levels of scale cortisol levels were associated with  
 260 higher growth (Fig. 2B, C, F) when scale cortisol was below *ca.*  $1 \mu\text{g.kg}^{-1}$  (following back

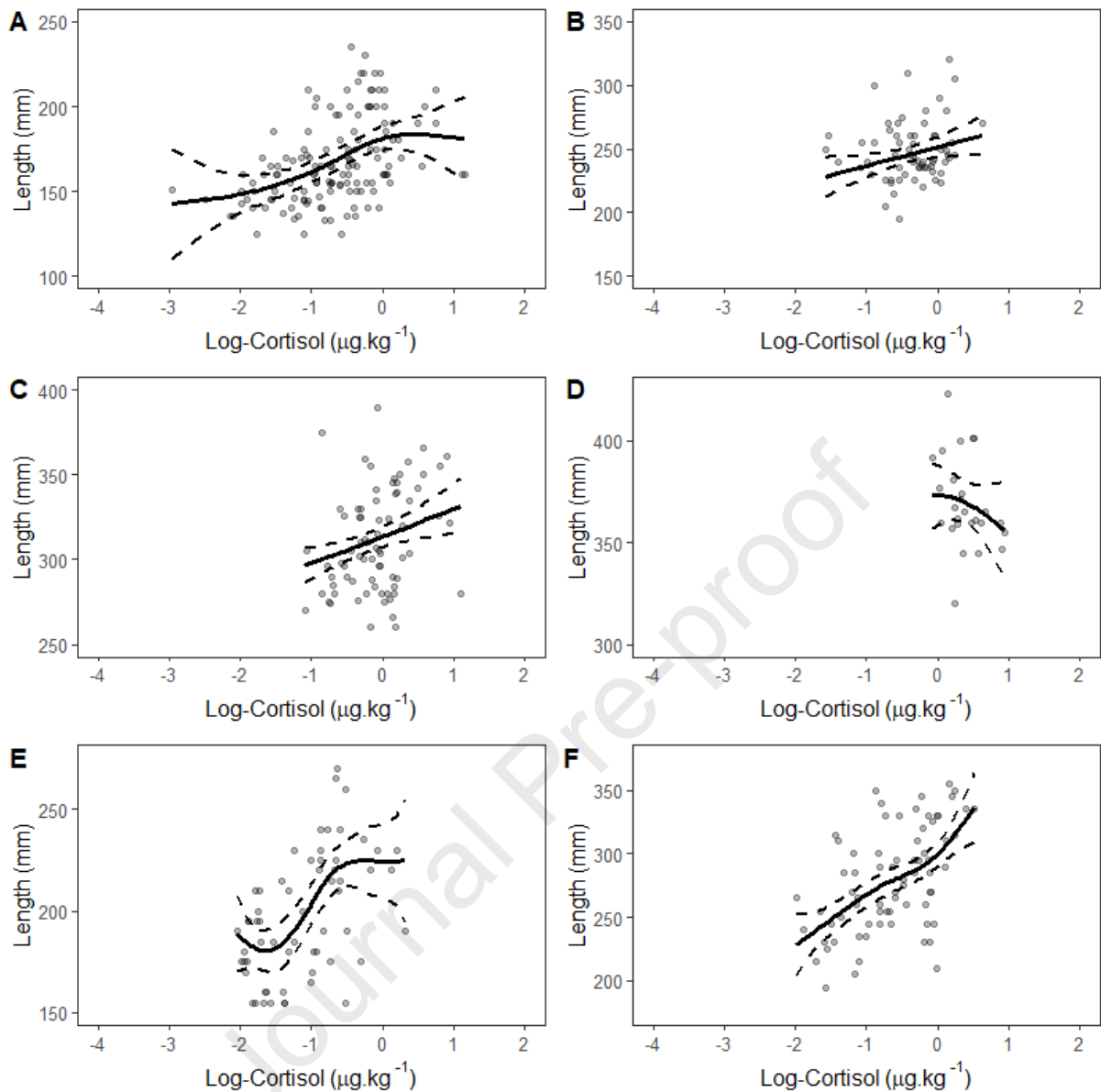
261 transformation). Fish with scale cortisol levels beyond this level had a lower growth in some  
 262 age-year combinations (Fig. 2A, D, E; Table A.3) but not others indicating a clear context  
 263 dependence of this non-linear relationship.

264

265 Table 2. Relative performance of models testing the effect of individuals' scale cortisol  
 266 concentration on their growth. Age and years were used as cofactors to account for systematic  
 267 differences in length and cortisol. The smoothing parameters were estimated for each year,  
 268 age and age-year combinations ('Age\*Year'). Table entries: number of degrees of freedom  
 269 (df), adjusted R-squared (Adj. R<sup>2</sup>), Akaike's Information Criterion (AIC), and differences in AICc  
 270 values relative to the best model ( $\Delta$ AIC).

271

	Factors	Smoothing	Df	Adj. R <sup>2</sup>	Deviance	AIC	$\Delta$ AIC
272	Age+Year	Age*Year	33.44	0.878	0.884	6634.3	0.0
273	Age*Year	Age*Year	42.46	0.880	0.886	6636.0	1.8
	Age	Age*Year	40.32	0.878	0.884	6645.0	10.8
274	Age*Year	Year	25.45	0.875	0.879	6645.7	11.5
275	Age+Year	Year	17.67	0.873	0.876	6651.2	17.0
	Age*Year	Age	24.85	0.874	0.878	6652.0	17.8
276	Age+Year	Age	17.25	0.870	0.872	6668.0	33.7
277	Age	Year	15.75	0.862	0.865	6704.9	70.6
	Year	Age*Year	52.30	0.859	0.869	6756.2	121.9
	Age	Age	14.81	0.842	0.845	6800.4	166.2
	Year	Age	16.01	0.422	0.434	7724.1	1,089.8
	Year	Year	12.53	0.359	0.368	7794.7	1,160.5



278

279 Figure 2. Estimated smooth curves of the effect of scale cortisol concentrations (log-  
 280 transformed) on the growth of sea bass juveniles. Smooth curves were estimated across  
 281 nurseries for each age in each year and are represented with their 95% confidence intervals.  
 282 Panels present the age-year combinations with significant effects: Age 1 in 2019 (Panel A),  
 283 Age 2 in 2019 (Panel B), Age 3 in 2019 (Panel C), Age 4+ in 2019 (Panel D), Age 2 in 2020  
 284 (Panel E), Age 3 in 2020 (Panel F). The same x-axes were used to facilitate the comparison  
 285 of scale cortisol levels in the different age and year categories (y-axes differ to facilitate the  
 286 representation of the effects).

## 287 4. DISCUSSION

288

289 Several studies have validated the usefulness of scale cortisol as a quantitative biomarker of  
290 chronic stress in the lab (Aerts et al., 2015; Laberge et al., 2019; Samaras et al., 2021) but to  
291 date the application of this method to wild fish has been limited. In wild sea bass juveniles, we  
292 found that the sampling year had by far the greatest effect, scale cortisol levels being lowest  
293 in 2017 and 2018 but substantially higher in 2019 and remained high in 2020 (an effect  
294 consistent across ages and nurseries). Even though sea bass juveniles were not sampled in  
295 Gironde in 2017 and 2018, the high scale cortisol levels in all ages measured in 2019 and 2020  
296 (significantly higher than those observed in Seine) in this nursery supports strongly the idea  
297 that sea bass juveniles responded to a very broad scale of stress factors. As the production of  
298 cortisol and its subsequent incorporation in growing ontogenetic scales can be due to a wide  
299 variety of environmental factors (e.g. salinity, oxygenation, temperature, illnesses, predation)  
300 it is not yet possible to directly test for a specific effect and, as such, we discuss three potential  
301 non-exclusive effects: chemical contaminations, river flow, and water temperature. Firstly, it is  
302 unlikely that pollution or chemical contaminants (i.e. trace metal elements or organic  
303 contaminants) would have led to such a consistent broad-scale increase in scale cortisol as  
304 each nursery has a specific contamination profile (Lebigre et al., 2022). For instance, Gironde  
305 is characterised by high levels of cadmium, DDTs, and dieldrin (Claisse, 1989; Lanceleur et  
306 al., 2011), Loire by high levels of lead and vanadium (Claisse, 1989; Couture et al., 2010), and  
307 Seine by high levels of silver and polychlorinated biphenyls (Chiffolleau et al., 2005). As these  
308 contaminants have different origins, it is unlikely that they would increase simultaneously in  
309 the three estuaries. Secondly, as cortisol mediates fish acclimation to salinity, changes in river  
310 flow (and salinity levels) might influence the variance in scale cortisol. This is however unlikely  
311 as the main flooding events in France occurred in May-June 2016 and February 2018. These  
312 floods would have influenced the scale cortisol levels of fish sampled in summer 2017 and  
313 2018 but these had substantially lower scale cortisol levels than those collected in 2019 and  
314 2020. Moreover, sea bass juveniles move along the estuary to adjust the salinity level of their

315 habitat, a behavioural adjustment that they undertake daily with tides (Roy et al., 2022), which  
316 might explain that flooding is a relatively weak stress factor. Finally, rapid increases in water  
317 temperature and/or prolonged exposures to high (or low) temperatures are well-known  
318 stressors to fish (e.g. Hanke et al., 2019), and may explain the large-scale increase in scale  
319 cortisol that we observed. Indeed, two heatwaves took place in July-August 2018 (an  
320 intermediate intensity but long lasting; Bastos et al., 2020) and two very intense heatwaves  
321 took place in June and July 2019 (Vautard et al., 2020). These heatwaves might have induced  
322 a prolonged and chronic stress response, that were observed in samples collected in the  
323 following summers (2019 and 2020) due to the time needed for the growing scales to  
324 incorporate cortisol (days to weeks; Gormally & Romero, 2020). It has already been shown  
325 experimentally that scale cortisol can reliably reflect chronic thermal stress (Goikoetxea et al.,  
326 2021) and that fish exposed to mild and gradual increasing temperatures have an increase in  
327 scale cortisol levels (Hanke et al., 2019). Clearly, only a longer time series would enable to  
328 quantify the relative contribution of multiple stress factors to the variance in scale cortisol in  
329 the wild and enable us to identify the factors that might underpin such large temporal variation  
330 in sea bass scale cortisol.

331

332 The second most important factor explaining the variance in scale cortisol levels in sea bass  
333 juveniles was their age: scale cortisol increased in a consistent way across nurseries and  
334 cohorts from one age class to another. Even during the steep increase in scale cortisol during  
335 2018-2019, the older age classes had consistently higher scale cortisol than their younger  
336 counterparts. This result can be explained by two non-exclusive processes: an accumulation  
337 of stressful events (and as such a longer period of cortisol production) with age and/or the  
338 gradual adaptation of older fish to seawater. Cortisol is progressively incorporated in growing  
339 scales and even though a previous study mentioned a potential clearance (Laberge et al.,  
340 2019) multiple studies have shown that scale cortisol accurately reflects medium to very long-  
341 term history of individuals' stress levels (Carbajal et al., 2019; Samaras et al., 2021). Estuaries  
342 are considered as naturally stressful areas because of the high variations of their

343 environmental characteristics relative to their neighbouring coastal and marine areas (e.g.  
344 oxygen, temperature, pH, turbidity, and salinity; Hughes et al., 2015; Le Guen et al., 2019;  
345 Vasconcelos et al., 2007). Species living in these habitats are however well-adapted to such  
346 variations and experimental studies have shown that fish stress responses can decline as they  
347 age and acclimate to the stress factors they are exposed to (Barton & Schreck, 1987; Maule  
348 & Schreck, 1991). Therefore, the increase in scale cortisol with age in sea bass juveniles  
349 suggests that older fish that have been gradually changing their habitat towards the estuaries  
350 mouth, did not really acclimate to stress factors, and that these changes in habitat might be  
351 associated with the encounter of new stress factors. Furthermore, sea bass juveniles  
352 progressively move towards the mouth of the estuaries and it is well established that cortisol  
353 mediates the acclimation of teleost fish to increasing salinity as it increases ions' secretion  
354 from gills by stimulating the proliferation and growth of gill chloride cells (McCormick, 2001).  
355 As changes in salinity also act as a stress factor, it might be difficult to disentangling the effect  
356 of more stressful events as fish age and changes in salinity but these results clearly show that  
357 understanding factors underpinning the variance in scale cortisol requires to account for age-  
358 and/or experience-specific effects.

359  
360 Finally, we found a slight difference in scale cortisol levels between nurseries, with sea bass  
361 juveniles sampled in Gironde and Loire having slightly higher scale cortisol levels than those  
362 sampled in Seine. This effect is consistent with other studies showing that the productivity and  
363 quality of nurseries may differ (Amara, Meziane, Gilliers, Hermel, & Laffargue, 2007; Courrat  
364 et al., 2009; Hughes et al., 2015; Vasconcelos et al., 2007). For instance, the levels of  
365 anthropogenic activity was consistent with differences in key phenotypic parameters (growth,  
366 body condition) in common sole (*Solea solea*; Amara et al., 2007), contamination indices were  
367 related to species density and richness (Courrat et al., 2009), and climatic variations influenced  
368 nursery productivity (Hughes et al., 2015). A previous study has also shown that scale cortisol  
369 levels could differ substantially among sampling sites at a relatively small scale (Hanke et al.,  
370 2020). We do not yet have information regarding the productivity of these estuaries for sea



371 bass juveniles, measurements of juvenile abundances being currently developed (Le Goff et  
372 al., 2017; Roy, Lebigre, Drogou, & Woillez, 2022). As sea bass juveniles sampled in these  
373 estuaries have different contamination profiles (Lebigre et al., 2022), some of them could have  
374 acted as additional stress factors, while others would not (Bechshøft et al., 2012; Brodeur et  
375 al., 1997). Further studies are therefore clearly needed to identify the processes underpinning  
376 such differences among nurseries in fish chronic stress levels.

377  
378 The overall scale cortisol levels that we have measured are broadly consistent and in the lower  
379 range of previous studies measuring this biomarker in wild fish. With a median of  $0.156 \mu\text{g.kg}^{-1}$ ,  
380 wild sea bass juveniles have similar scale cortisol levels to those of wild milkfish (*Chano*  
381 *chano*) across 4 sampling sites *ca.*  $0.04\text{-}0.12 \mu\text{g.kg}^{-1}$  (Hanke et al., 2020), but lower than those  
382 reported in *Neolamprologus pulcher* males and females ( $1.49$  and  $3.50 \mu\text{g.kg}^{-1}$  across fish for  
383 which cortisol could be measured; Culbert et al., 2021), yellow-fin tuna (*Thunnus albacares*;  
384  $1.65 \mu\text{g.kg}^{-1}$ ; Roque d'orbcastel et al., 2021), skipjack tuna (*Katsuwonus pelamis*;  $4.75 \mu\text{g.kg}^{-1}$ ;  
385 Roque d'orbcastel et al., 2021) and two Catalan chub populations (*Squalius laietanus*; *ca.*  
386  $32 \mu\text{g.kg}^{-1}$  and  $30 \mu\text{g.kg}^{-1}$  Carbajal et al., 2019). These interspecific differences can stem from  
387 many factors (e.g. life histories, stress histories, sampled ages, environmental variability,  
388 coping abilities), hence it might only be possible to understand these variations by measuring  
389 scale cortisol in other wild fish species. On a logarithmic scale, the greatest difference in scale  
390 cortisol levels between consecutive ages is  $2.176 \mu\text{g.kg}^{-1}$  (from  $-1.619$  to  $0.357 \mu\text{g.kg}^{-1}$  between  
391 the ages 3 and 4+ in Loire 2018-2019 on a logarithmic scale of base 10; Table A.1). This  
392 increase represents an increase by 2 orders of magnitude in scale cortisol levels. It is not yet  
393 possible to compare this maximum rate of increase to other wild fish species as previous  
394 studies lacked longitudinal data to quantify temporal changes in scale cortisol levels within  
395 cohorts. The low detection limit of the UPLC-MS/MS method used in our study ( $0.0001 \mu\text{g.kg}^{-1}$ )  
396 enabled us to measure very low scale cortisol levels for many sea bass juveniles, which in  
397 turns underpins to some extent the very large increase in scale cortisol levels that we have  
398 detected. Two experimental studies showed that fish exposed to warm temperatures had a 10-

399 fold increase in scale cortisol (Goikoetxea et al., 2021) and a 1.43-fold increase (Hanke et al.,  
400 2019). However, direct comparisons of these rates increases with our data is not really possible  
401 because (i) scale cortisol levels in the first experimental study were very high and might have  
402 dampened fish stress response to warm conditions ( $2,100 \mu\text{g}\cdot\text{kg}^{-1}$  for the control treatment in  
403 Goikoetxea et al., 2021), (ii) the second study aimed at measuring the effect of warming over  
404 very short periods (21 days, Hanke et al., 2019) and hence was unlikely to detect extremely  
405 high responses in scale cortisol levels, (iii) the acclimation of fish to laboratory conditions might  
406 have to changed in the regulation of their primary stress response, and (iv) we would need to  
407 measure in wild fish the effect of water temperature on the variance of scale cortisol levels  
408 while accounting for other stress factors (this would require longer time series).

409  
410 The differences in scale cortisol among ages and years had a strong influence on the  
411 relationship between individuals' growth and their scale cortisol level. Indeed, there was no  
412 significant effect across the 8 age-year classes in 2017 and 2018 (Table A.1), suggesting that  
413 sea bass juveniles had stress levels that were probably too low to induce any deleterious effect  
414 during these years. Conversely, there were clear relationships between scale cortisol and the  
415 growth of sea bass juveniles in 2019 and 2020 in which fish with higher scale cortisol levels  
416 had higher growth (Fig. 2A, B, C, E, F) but this relationship declined when fish had particularly  
417 high scale cortisol levels (Fig. 2A, E), and eventually the relationship became negative (Fig.  
418 2D). Obviously, there are many factors that influence fish growth (many of which cannot be  
419 estimated in the wild) and the absence of relationship in 2017 and 2018 suggests that external  
420 factors underpin substantially the magnitude (and shape) of this relationship. It is possible that  
421 during these particularly warm years, the metabolism of sea bass juveniles was accelerated  
422 leading faster growth but also higher stress. The plateau of this relationship probably reflects  
423 the deleterious effects of too high stress levels, consistent with the idea that there is an optimal  
424 stress level and that fish with elevated chronic stress levels do have lower growth (Korte,  
425 Koolhaas, Wingfield, & McEwen, 2005; McEwen & Wingfield, 2003). Such significant non-  
426 linear relationships between scale cortisol and growth in years when the overall level of scale

427 cortisol was high is a good example of the year, site, and age dependency of the relationship  
428 between stress levels and fitness (Bonier et al., 2009). This context-dependence is often put  
429 forward to explain the large variation in effect sizes between studies and, for instance, no  
430 significant difference in scale cortisol and fish size was recently found in tunas (Roque  
431 d'orbcastel et al., 2021).

432

433 There was no consistent decline in growth at very high cortisol levels suggesting that cortisol  
434 levels were either not yet high enough to induce a steep decline in growth or that the fish with  
435 very high cortisol levels have died further supporting the detrimental effect of long-term  
436 upregulated cortisol levels and subsequent increased mortality. As fish size is positively linked  
437 to their survival rate (Crossin, Cooke, Goldbogen, & Phillips, 2014; Gislason et al., 2010; Pauly,  
438 1980), fish with very high stress levels and low growth may have died before sampling. When  
439 sequential fitness components are considered separately, viability selection at an early  
440 (unmeasured) stage can lead to substantial underestimates of the magnitude of selection (the  
441 'missing fraction issue', e.g. Hadfield, 2008) and even change the direction observed selection  
442 gradients. Fully understanding the degree to which chronic stress is related to survival would  
443 therefore require estimating the chronic stress of tagged individuals and measure their short,  
444 medium and long-term survival rates. However, setting up such an investigation in a wild  
445 population would be very challenging, requiring a very large sampling effort, high  
446 recapture/recovery probability, and might prove impossible especially for the youngest age  
447 classes in which the decline in survival of highly stress fish might be the strongest. On the  
448 other hand, a large scale sampling campaign over an extended period would be more feasible.  
449 Similarly, cortisol production is often sex-specific (Idler & Freeman, 1968) but as gonads were  
450 not developed enough to enable us to accurately determine each individuals' sex, we cannot  
451 test the hypothesis that females have higher scale cortisol levels than males.

452

453 5. CONCLUSION

454 Overall, the consistent increase in scale cortisol with age in all nurseries and years, and the  
455 consistent increase in cortisol with time in two nurseries separated by *ca.* 300 km clearly  
456 indicates that scale cortisol can be used as a quantitative biomarker for broad- and local-scale  
457 constraints (chronic stress) in wild fish. We observed clear non-linear effects of scale cortisol  
458 on juveniles' growth but only in years in which scale cortisol was particularly high. This effect  
459 suggests that when environmental conditions induce high levels of chronic stress, reflected in  
460 high scale cortisol levels, they might also impair fish growth and hence potentially other fitness  
461 components that we cannot yet measure (survival, reproductive investment). Longer-term data  
462 are clearly needed to enable us to quantify the relative contribution of various environmental  
463 variables to the variance in scale cortisol and pinpoint the environmental factor(s) causing  
464 chronic stress in juvenile sea bass.

465

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478

#### 479 AUTHOR CONTRIBUTIONS:

480 CL and JA conceived the ideas and designed methodology; MD and RLG led the overall data collection,  
481 CL, MW, HR, JM, MD, RLG, AS, JH, MV carried out scale cortisol analyses; CL, MW, and JM analysed

482 the data; CL and JA led the writing of the manuscript. All authors contributed critically to the drafts and  
483 gave final approval for publication.

484

#### 485 DATA AVAILABILITY STATEMENT

486 Datasets used in these analyses are available in the Archimer repository.

487

#### 488 CONFLICT OF INTEREST:

489 None

490

Journal Pre-proof

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709 Table A.1. Summary table of the mean scale cortisol concentrations (log-transformed) per  
 710 year, sampling site ('Nursery'), age, and cohorts. Standard deviations (SD) and standard errors  
 711 (SE) are provided (sample sizes). Sea bass juveniles were sampled in three large nurseries of  
 712 France's western coast (Gironde, Loire, and Seine). Fish aged over 4 years old were pooled  
 713 in a single class and no survey took place in Gironde in 2017 and 2018.

Year	Nursery	Age	N	Cohort	Cortisol concentration	SD	SE
2017	Loire	1	19	2016	-1.544	0.325	0.075
2017	Loire	2	9	2015	-1.261	0.340	0.113
2017	Loire	3	20	2014	-1.180	0.509	0.114
2017	Loire	4+	11	2013	-0.707	0.415	0.125
2017	Seine	1	20	2016	-1.886	0.748	0.167
2017	Seine	2	20	2015	-1.431	0.715	0.160
2017	Seine	3	20	2014	-0.873	0.872	0.195
2018	Loire	1	19	2017	-2.029	0.356	0.082
2018	Loire	2	19	2016	-1.796	0.425	0.098
2018	Loire	3	5	2015	-1.818	0.426	0.190
2018	Loire	4+	14	2014	-0.822	0.542	0.145
2018	Seine	1	15	2017	-2.386	0.567	0.142
2018	Seine	2	15	2016	-2.206	0.308	0.082
2018	Seine	3	16	2015	-1.619	0.624	0.156
2018	Seine	4+	17	2014	-1.287	0.642	0.156
2019	Gironde	1	62	2018	-0.319	0.511	0.065
2019	Gironde	2	10	2017	-0.342	0.532	0.168
2019	Gironde	3	9	2016	0.069	0.446	0.149
2019	Gironde	4+	3	2015	0.387	0.293	0.169
2019	Loire	1	36	2018	-0.658	0.723	0.120
2019	Loire	2	32	2017	-0.382	0.349	0.062
2019	Loire	3	19	2016	-0.142	0.474	0.109
2019	Loire	4+	4	2015	0.357	0.368	0.184
2019	Seine	1	39	2018	-1.285	0.599	0.096
2019	Seine	2	23	2017	-0.336	0.521	0.109
2019	Seine	3	46	2016	-0.091	0.464	0.068
2019	Seine	4+	19	2015	0.396	0.261	0.060
2020	Gironde	1	3	2019	-2.291	0.178	0.103
2020	Gironde	2	22	2018	-1.370	0.738	0.157
2020	Gironde	3	27	2017	-0.825	0.629	0.121
2020	Gironde	4+	8	2016	-0.390	0.638	0.225
2020	Loire	1	11	2019	-1.555	0.380	0.114
2020	Loire	2	20	2018	-0.977	0.333	0.074
2020	Loire	3	25	2017	-0.383	0.439	0.088
2020	Loire	4+	4	2016	-0.076	0.273	0.136
2020	Seine	1	4	2019	-1.258	0.556	0.278
2020	Seine	2	18	2018	-0.966	0.645	0.152
2020	Seine	3	20	2017	-0.699	0.675	0.151
2020	Seine	4+	18	2016	-0.296	0.584	0.138



714 Table A.2. Output summary of the two best models explaining the variance in scale cortisol  
 715 across sampling nursery, sampling years, and sea bass juveniles' age. These two models have  
 716  $\Delta AICc$  below 2. Differences between levels of each of the categorical variables are also  
 717 provided ( $\beta$ ) with their associated standard error (SE), t-ratio (t), and their p-value ( $P$ )  
 718

Model	Variable	$X^2$	df	$P$	Levels	$\beta$	SE	t	$P$
Age+Year	Age	174.8	3	< 0.001	-	-	-	-	-
					Age 1-Age 2	-0.308	0.062	-4.940	< 0.001
					Age 1-Age 3	-0.703	0.073	-9.704	< 0.001
					Age 1-Age 4+	-1.165	0.092	-12.656	< 0.001
					Age 2-Age 3	-0.395	0.064	-6.191	< 0.001
					Age 2-Age 4+	-0.857	0.084	-10.243	< 0.001
					Age 3-Age 4+	-0.462	0.076	-6.073	< 0.001
	Year	567.3	3	< 0.001	-	-	-	-	-
					2017-2018	0.567	0.079	7.197	< 0.001
					2017-2019	-0.981	0.075	-13.005	< 0.001
					2017-2020	-0.288	0.090	-3.195	0.008
					2018-2019	-1.548	0.069	-22.360	< 0.001
					2018-2020	-0.855	0.081	-10.532	< 0.001
					2019-2020	0.693	0.060	11.527	< 0.001
Age+Year+Nursery	Age	213.3	3	< 0.001	-	-	-	-	-
					Age 1-Age 2	-0.315	0.061	-5.139	< 0.001
					Age 1-Age 3	-0.711	0.068	-10.525	< 0.001
					Age 1-Age 4+	-1.164	0.085	-13.742	< 0.001
					Age 2-Age 3	-0.396	0.062	-6.389	< 0.001
					Age 2-Age 4+	-0.849	0.079	-10.707	< 0.001
					Age 3-Age 4+	-0.453	0.075	-6.077	< 0.001
	Year	575.6	3	< 0.001	-	-	-	-	-
					2017-2018	0.553	0.078	7.120	< 0.001
					2017-2019	-0.985	0.073	-13.575	< 0.001
					2017-2020	-0.282	0.085	-3.303	0.006
					2018-2019	-1.538	0.069	-22.419	< 0.001
					2018-2020	-0.835	0.079	-10.532	< 0.001
					2019-2020	0.704	0.059	11.873	< 0.001
	Nursery	4.5	2	0.1051	-	-	-	-	-
					Gironde-Seine	0.135	0.093	1.447	0.343
					Gironde-Loire	-0.012	0.094	-0.125	0.991
	Seine-Loire	-0.146	0.074	-1.979	0.152				

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721 Table A.3. Parameters of the smooth terms relating cortisol to fish growth for each age class  
 722 and year (best generalised additive model). Table entries: edf, effective degrees of freedom;  
 723 F, F-tests on the smooth terms; P, p-value (significance threshold: 0.05). An asterix denotes  
 724 significant effects.

725

Smooth term	edf	F	P
s(cortisol):Age1:2017	1.67	2.35	0.081
s(cortisol):Age2:2017	1.00	1.12	0.290
s(cortisol):Age3:2017	1.00	0.45	0.504
s(cortisol):Age4+:2017	2.21	3.62	0.020*
s(cortisol):Age1:2018	1.35	1.76	0.099
s(cortisol):Age2:2018	1.00	1.16	0.282
s(cortisol):Age3:2018	1.00	0.88	0.349
s(cortisol):Age4+:2018	1.00	1.89	0.170
s(cortisol):Age1:2019	2.78	8.07	< 0.001*
s(cortisol):Age2:2019	3.40	3.64	0.008*
s(cortisol):Age3:2019	1.00	12.45	< 0.001*
s(cortisol):Age4+:2019	1.88	5.52	0.004*
s(cortisol):Age1:2020	1.44	0.38	0.580
s(cortisol):Age2:2020	2.93	8.76	0.001*
s(cortisol):Age3:2020	3.12	14.24	< 0.001*
s(cortisol):Age4+:2020	2.59	1.33	0.252

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## Highlights

### TEMPORAL VARIATIONS IN SCALE CORTISOL INDICATE CONSISTENT LOCAL- AND BROAD-SCALE CONSTRAINTS IN A WILD MARINE TELEOST FISH

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- Environment's effect on individuals' growth is mediated by their chronic stress
- We measured scale cortisol in juvenile sea bass aged 1-4+ during 2017-2020
- Scale cortisol increased substantially in 2019 and 2020 in all nurseries/age
- High scale cortisol was related to lower growth only in 2019 and 2020
- Scale cortisol might reflect local to broad-scale environmental constraints

SCALE CORTISOL TO QUANTIFY ENVIRONMENTAL CONSTRAINTS ON A WILD MARINE  
TELEOST FISH

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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