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   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 (CNPMEM, 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 (± 0.2 g), and liver weight (± 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 ruptor (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

7

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 µg.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 µg.kg^{-1} with a median of 0.169 µg.kg^{-1} (IQR: 0.029-0.642 µg.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 (ΔAIC = 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   (ΔAICc > 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 (ΔAICc), model weight (w_i). Interaction terms are noted

240 with ':' between two main effects.

Parameters	K	logLik	AICc	ΔAICc	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

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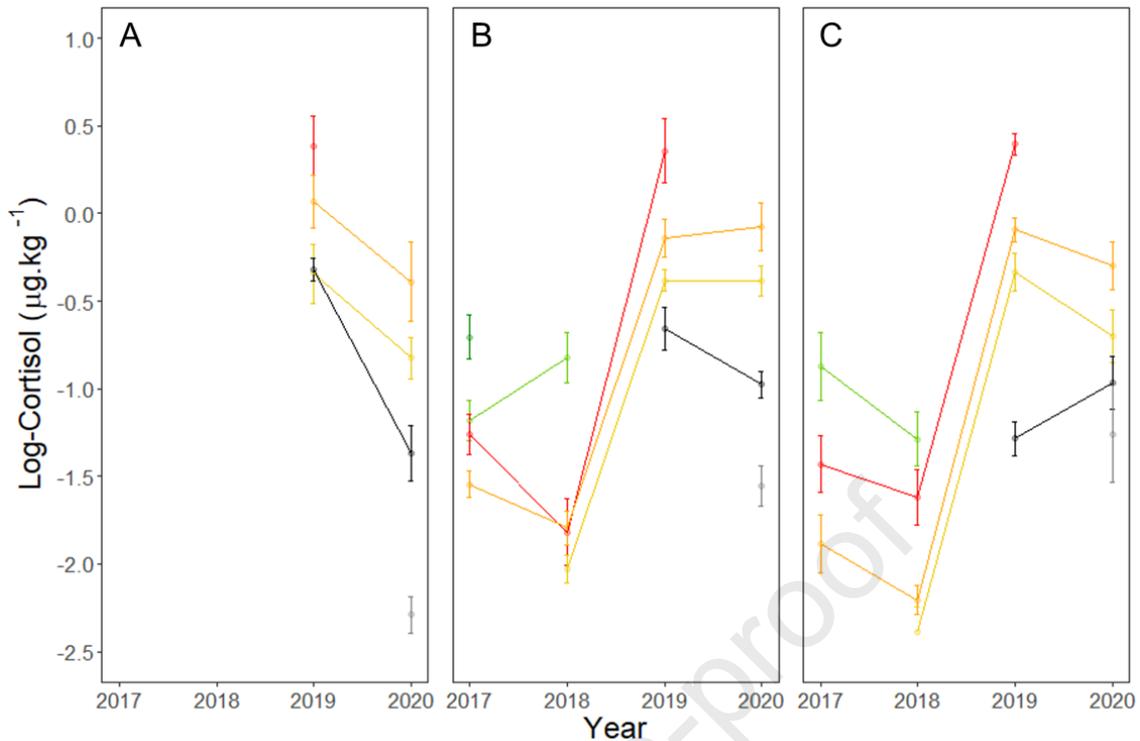
244

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 µg.kg^{-1} (following back

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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^2), Akaike's Information Criterion (AIC), and differences in AICc

270 values relative to the best model (ΔAIC).

271

Factors	Smoothing	Df	Adj. R^2	Deviance	AIC	ΔAIC
Age+Year	Age*Year	33.44	0.878	0.884	6634.3	0.0
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
Age*Year	Year	25.45	0.875	0.879	6645.7	11.5
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
Age+Year	Age	17.25	0.870	0.872	6668.0	33.7
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

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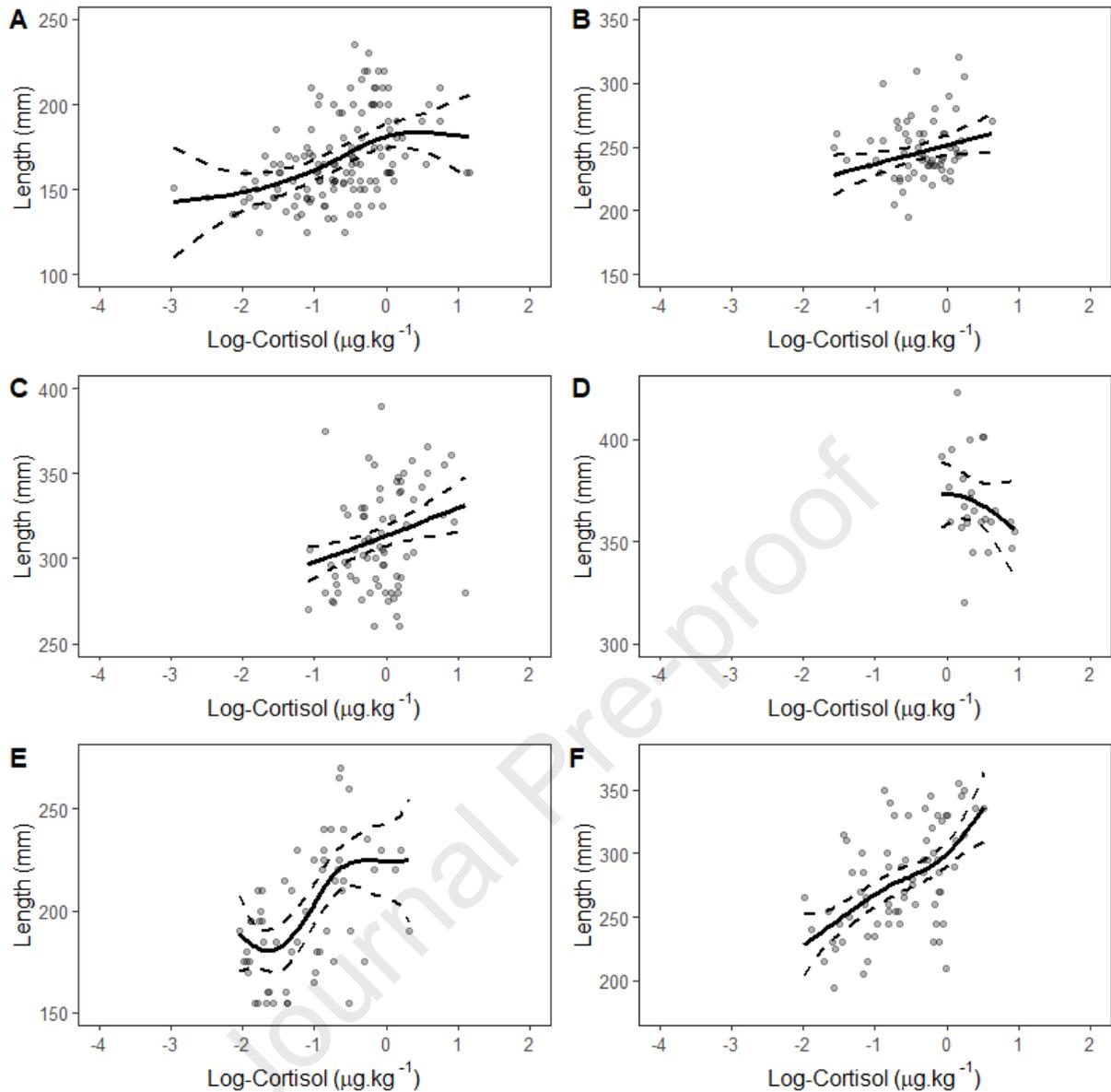
Figure 2. Estimated smooth curves of the effect of scale cortisol concentrations (log-transformed) on the growth of sea bass juveniles. Smooth curves were estimated across nurseries for each age in each year and are represented with their 95% confidence intervals. Panels present the age-year combinations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

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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

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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 µg.kg^{-1}

380   ^{1}, wild sea bass juveniles have similar scale cortisol levels to those of wild milkfish (*Chano*

381   *chano*) across 4 sampling sites *ca.* 0.04-0.12 µg.kg^{-1} (Hanke et al., 2020), but lower that those

382   reported in *Neolamprologus pulcher* males and females (1.49 and 3.50 µg.kg^{-1} across fish for

383   which cortisol could be measured; Culbert et al., 2021), yellow-fin tuna (*Thunnus albacares*;

384   1.65 µg.kg^{-1}; Roque d'orbcastel et al., 2021), skipjack tuna (*Katsuwonus pelamis*; 4.75 µg.kg^{-1}

385   ^{1}; Roque d'orbcastel et al., 2021) and two Catalan chub populations (*Squalius laietanus*; *ca.*

386   32 µg.kg^{-1} and 30 µg.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 µg.kg^{-1} (from -1.619 to 0.357 µg.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 µg.kg^{-1}

396   ^{1}) 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 µg.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

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

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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 ΔAICc below 2. Differences between levels of each of the categorical variables are also

717 provided (β) with their associated standard error (SE), t-ratio (t), and their p-value (*P*)

718

Model	Variable	X^2	df	*P*	Levels	β	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: