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1 **An integrative perspective on fish health: environmental and anthropogenic**
2 **pathways affecting fish stress**

3

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21 **ABSTRACT**

22 Multifactorial studies assessing the cumulative effects of natural and anthropogenic stressors
23 on individual stress response are crucial to understand how organisms and populations cope
24 with environmental change. We tested direct and indirect causal pathways through which
25 environmental stressors affect the stress response of wild gilthead seabream in Mediterranean
26 coastal lagoons using an integrative PLS-PM approach. We integrated information on 10
27 environmental variables and 36 physiological variables into seven latent variables reflecting
28 lagoons features and fish health. These variables concerned fish lipid reserves, somatic
29 structure, inorganic contaminant loads, and individual trophic and stress response levels. This
30 modelling approach allowed explaining 30% of the variance within these 46 variables
31 considered. More importantly, 54% of fish stress response was explained by the dependent
32 lagoon features, fish age, fish diet, fish reserve, fish structure and fish contaminant load latent
33 variables included in our model. This integrative study sheds light on how individuals deal
34 with contrasting environments and multiple ecological pressures.

35

36

37 Key words: Ecosystem, PLS-PM, Eco-physiology, Direct and indirect effects, Fish health,
38 Stress

39

40

41 INTRODUCTION

42 In natural ecosystems, organisms are subject to a variety of environmental pressures
43 that act simultaneously and vary over time and space. Understanding the consequences of
44 environmental variation on individual health, and their long-term consequences on population
45 health and dynamics, requires deciphering the complex interactions between environmental
46 pressures (natural and anthropogenic) and their causal effects on organisms (Doney et al.,
47 2012; Portner and Farrell, 2008). In complex ecosystems, ecological studies often progress in
48 small steps, identifying and testing the effects of a few handpicked variables on individual
49 organism health and fitness (Doney et al., 2012; Portner and Farrell, 2008). However, where
50 interactions among multiple ecological pressures (both intrinsic and extrinsic to the organism)
51 may generate interactive effects, single-stressor based models reach a limit: the resulting
52 global impact of independent environmental pressures on individual organisms might either
53 exceed (i.e. synergism) or fall below (i.e. antagonism) their expected additive effects (Côté et
54 al., 2016; Crain et al., 2008). Therefore, there is an urgent need for multifactorial studies
55 where the cumulative effect of multiple environmental pressures on individual organisms is
56 assessed (Brosset et al., 2021; Rosa and Seibel, 2008). This can be achieved through
57 integrative modelling approaches that consider both the putative causal direct and indirect
58 pathways through which environmental pressures may affect individual health and fitness.

59 Such approaches should be particularly useful in transitional ecosystems, such as
60 coastal lagoons, where organisms are subject to a high diversity of environmental pressures.
61 Together with estuaries, coastal lagoons form part of a continuum between continental and
62 aquatic ecosystems and are consequently exposed to highly variable hydrological conditions
63 (Pérez-Ruzafa et al., 2011). Situated at the interface between open sea and land, these areas of
64 high biological productivity receive high inputs of organic matter and nutrients of natural and
65 human origin (e.g. agriculture and urban run-offs), but also act as reservoirs for toxic

66 compounds and disruptors, such as pollutants (Losso and Ghirardini, 2010; Lotze, 2006;
67 Nixon et al., 2007). In such contexts, identifying favourable habitats for a given species
68 and/or life history stage, is faced with the challenging task of assessing how individual
69 organisms cope with the array of interacting environmental factors which they are subjected
70 to in contrasting environments. One way of doing so is to focus on the cumulative effects of
71 multiple natural and anthropogenic stressors on individual physiological processes (Todgham
72 and Stillman, 2013), as these are tightly related to individual performance and fitness
73 (Bryndum-Buchholz et al., 2019; Purves et al., 2013; Williams et al., 2008).

74

75 *Stress response as an integrative measure of individual health*

76 A key physiological system involved in regulating individual homeostasis is the
77 hypothalamic–pituitary–adrenal (HPA; or interrenal, e.g. in fish) axis, a highly conserved
78 function across vertebrates (Denver, 2009). Subjected to a stressor (external – for instance
79 predation, or internal – for instance fasting), the activation of the HPA axis ultimately leads to
80 the secretion of glucocorticoid (GC) hormones by the adrenal cortex (Wingfield and Romero,
81 2011), whose actions mediate changes in individual energy balance (e.g. mobilisation of
82 energy stores; immunity; cognition; visual acuity; and behaviour, Munck et al. 1984, Johnson
83 et al. 1992). This initial cascade of physiological and behavioural changes enables the
84 organism to cope with acute stressors by mobilising adequate bodily functions, while
85 concurrently inhibiting non-essential functions (e.g. reproduction, digestion) (Wingfield and
86 Romero, 2011). Thus, a commonly used marker of individual stress response is the measure
87 of circulating GC concentrations (Goikoetxea et al., 2021; Harris, 2020; Sadoul and Geffroy,
88 2019). Yet, since the primary function of GCs is to promote adaptive responses to changing
89 environments, GC levels alone are not sufficient to determine whether individuals' coping

90 capacities have been overwhelmed by environmental stressors (Boonstra, 2013; Boonstra et
91 al., 2007; MacDougall-Shackleton et al., 2019).

92 Thus, in addition to increased glucocorticoid levels, physiologically stressed
93 individuals often exhibit downstream consequences associated with the long-term
94 overstimulation of emergency responses (Romero, 2004). For instance, individual oxidative
95 stress increases under acute as well as chronic stress and has been related to glucocorticoid
96 levels in many animals (Costantini et al., 2011; Lemonnier et al., 2022). Changes in
97 individual immune profiles may also present other downstream metric useful to characterize
98 chronic stress. Chronic stress loads that exceed the normal reactive scope of individuals are
99 often associated with immunosuppressive effects (see Martin 2009; Table 1 in Romero et al.
100 2009) such as down-regulation of inflammatory responses (Cohen et al., 2012) or changes in
101 leukocyte profiles (Davis et al., 2008). Taken together, glucocorticoid levels, oxidative stress
102 status and immune function may present an integrative view of individual exposure to acute
103 and chronic stress and provide a good overview of individual health and how individuals deal
104 with their environment.

105

106 *Factors affecting individual health and the case of aquatic ecosystems*

107 Extrinsic (e.g. predation, parasitism, microclimate, contaminant) and intrinsic (e.g. individual
108 age, body condition, life history stage or genetic) factors may affect organism stress through
109 both direct and indirect pathways. In aquatic ecosystems, limited oxygen (Wajsbrov et al.,
110 1991), extreme osmotic (Laiz-Carrión et al., 2005; Tandler et al., 1995) or temperature
111 (Heather et al., 2018) conditions have direct impacts on organism's immune function (Cuesta
112 et al., 2005) and oxidative status (Madeira et al., 2016, 2013). Metal contaminants
113 accumulated in an organism also have direct long-term consequences on anti-oxidant
114 depletion (Médale et al., 2005; Saera-Vila et al., 2009), enhancing oxidative stress (Benhamed

115 et al., 2016; Mourente et al., 2002; Soares et al., 2008) and impairing immune function
116 (Cerezuela et al., 2016; Guardiola et al., 2016, 2015). Similarly, individual age or experience
117 also directly affects the way organisms cope with given stressors; older individuals often
118 coping more efficiently with stressors owing to past experience, until late life senescence
119 (Barcellos et al. 2012, Navaratnarajah and Jackson 2013). For instance, age- and species-
120 specific changes in bio-accumulation or sensitivity to specific contaminants are thought to
121 evolve: metal contaminants such as mercury (Hg) shows bio-accumulative patterns over life
122 within and across species (Ourgaud et al., 2018), whereas others are detoxified or diluted
123 through time (Lee et al., 1998). Indeed some contaminant are known to be diminished during
124 ontogenetic development (e.g. passed within the eggs, Johnson et al., 2020) or due to a shift in
125 the diet while growing, therefore lowering exposition (Lee et al., 1998). Moreover
126 contaminants may induce reduced stress response often underlying difficulties to deal with
127 these stressors (Hontela and Fortin, 1995; Tan et al., 2009). In the other hand some essential
128 trace elements (e.g. Cu, Zn) are necessary in small amount to insure vital physiological
129 functions (Wood et al., 2012) but have detrimental effect in high concentrations (Minghetti et
130 al., 2008; Oliva et al., 2007; Shuhaimi-Othman et al., 2015). Such relationships urge the use
131 of several markers of individual health and insist on the necessity to consider both positive
132 and negative relationship between contaminant and stress response.

133

134 Besides these direct effects, extrinsic and intrinsic factors also influence individual
135 stress response through indirect pathways. In particular, environment-driven changes (e.g.
136 temperature, light, salinity, pH) affecting lower trophic levels of the food web, can also affect
137 higher trophic levels indirectly by modifying prey availability (Chassot et al., 2010).
138 Assessing variation in individual diets may therefore present a convenient tool for tracking
139 indirect effects on individual energy stored, growth and stress response. Organic matter inputs

140 are central to aquatic ecosystem functioning, affecting primary production (phytoplankton vs.
141 aquatic angiosperms in eutrophic vs. oligotrophic conditions), as well as higher trophic levels
142 (i.e. affecting the nature of reserves fish assimilate as well as their growth capacities; Escalas
143 et al., 2015; Isnard et al., 2015; Le Fur et al., 2019). For instance, whereas fish grow faster at
144 intermediate salinities, salinity also has complex interactions with temperature and oxygen
145 concentrations, and the consequences on food intake and food conversion are species-
146 dependant (Bœuf and Payan, 2001). Increasing salinity also limits metal contaminant
147 accessibility in animal (Ifremer, 2009; Lee et al., 1998) and bioaccumulation most probably
148 due to osmo-regulation modulation (Lee et al., 1998; Wright, 1995) but biodisponibility in the
149 water and sediments tend to improve (Zhao et al., 2013).

150

151 *Study aim and choice of a relevant fish model*

152 As a case study, we focused on the ecophysiology of gilthead seabream (*Sparus*
153 *aurata*) in the Gulf of Lions (Mediterranean Sea), which migrate yearly between coastal
154 lagoons (for growth and development) and the open sea (for reproduction) during its juvenile
155 and adult stages (Mercier et al., 2012). The physiology of gilthead seabreams has been well
156 studied and physiological markers of chronic stress, including high cortisol and oxidative
157 stress and decreased immune function, have been identified (Salati et al., 2016; Tort et al.,
158 1998; Vera et al., 2014). As a ectothermic species, Seabream growth and movement are
159 affected by environmental changes in temperature (Heather et al., 2018; Madeira et al., 2016;
160 Mignucci et al., 2021). In addition, the environmental conditions of coastal lagoons are known
161 to vary over short spatial scales, including in dissolved oxygen concentrations, eutrophication,
162 and contaminants known to cause oxidative stress and depress fish immune system
163 (Benhamed et al., 2016; Cerezuela et al., 2016).

164 Here, we focused on young seabreams (0-3 years old) captured in ten coastal lagoons,
165 at an age where lagoon conditions, including diet, have marked long-term consequences on
166 individual growth (Escalas et al., 2015; Isnard et al., 2015) and stress response (Médale et al.,
167 2005; Mourente et al., 2000). The early life period of fish is a critical period of growth and
168 development, associated with high mortality rates (Cushing and Horwood, 1994). To assess
169 the direct and indirect effects of environmental factors on individual physiology, stress
170 response and health, including synergic, agonistic or additive effects (e.g. cocktail effects
171 between contaminants; Celandier 2011), we used a Partial Least Squares-Path Modelling
172 (PLS-PM) approach. PLS-PM evaluates interactions through a combination of multiple linear
173 regressions and principal component Analyses (Wold, 1980; Esposito Vinzi et al., 2010;
174 Sanchez, 2013).

175 We expected contaminant loads to directly impair individual health. We further
176 expected abiotic and biotic environmental features (eutrophication, organic matter, salinity
177 and depth), as well as individual age to affect seabream growth and body condition (i.e. lipids
178 and protein content), through changes in their diet. Indeed eutrophication and organic matter
179 inputs are known to boost the entire trophic web as well as seabreams growth rate (Brehmer et
180 al., 2013; Escalas et al., 2015; Isnard et al., 2015). Salinity and organic matter contents play a
181 role in contaminant biodisponibility (Richards et al., 2001; Schwartz et al., 2004; Zhao et al.,
182 2013) and since Gilt-head seabreams is a necto-benthic species it feeds and uses bottom
183 habitats. Moreover the depth of the lagoon may play a role in hidden from predator, especially
184 early in life. Finally organisms allow energy to growth to reach maturity and reproduce as
185 early as possible but also to avoid predation. Ultimately this structural investment (hereafter
186 estimated by growth rate, protein content and structural lipids known as phospholipids) will
187 necessarily require energy for maintenance (structural cost, Stearns, 1989). When resources
188 exceed these cost, energy can be stored under the form of reserve lipid (Triacylglycerol,

189 Anedda et al., 2013; Queiros et al., 2019). Since fish condition is known to interact with
190 contaminant bio-accumulation, and therefore indirectly with stress response, the use of a
191 PLS-PM approach (see Fig. 1 for the general construct) and comparison of seven alternative
192 models, allowed for a comprehensive study into how environmental conditions, individual
193 condition and environmental contaminant affected fish health in coastal lagoons.

194

195 **METHODS**

196 **Environmental descriptors**

197 We monitored ten coastal lagoons (Arnel, Ayrolle, Gruissan, Ingril, Leucate, Or, Prévost,
198 Bages-Sigean, Thau, and Vic; latitude: from 48° 51' 18" N to 43° 34' 50" N; longitude from 3°
199 0' 21" E to 4° 2' 16" E; see Fig. 2) in the Gulf of Lions (France, NW Mediterranean Sea, Fig.
200 2). Lagoon morphological features included surface area (km²), maximum depth (m), and
201 hydrobiological characteristics of the water column (collected 1 m below the surface),
202 including salinity, dissolved inorganic nitrogen (DIN, μM), total nitrogen (μM), total
203 phosphorus (μM), phosphate (μM), chlorophyll *a* (μg.L⁻¹), dissolved oxygen concentration
204 (O₂, mg.L⁻¹), and Turbidity (NTU), as integrative descriptors of the environment at a lagoon
205 scale (ESM1). Those data were acquired via the OBSLAG network and the Water Framework
206 Directive (WFD) (Bouchoucha et al., 2019; Derolez et al., 2017). Data were collected
207 monthly from 2014-17 (time period corresponding to the age in years of the sampled
208 seabreams, see below) during summer (June, July, August), from a single station in smaller
209 lagoons (Arnel, Ayrolle, Gruissan, Ingril, Prévost, and Vic), two stations in intermediate sized
210 lagoons (Bages-Sigean, Leucate and Or), and six stations in the largest lagoon (Thau).

211 Linking lagoon morphometric and hydrobiological features to relevant biological
212 functions or mechanisms is not trivial and previous studies have addressed this issue (see

213 Derolez et al., 2020 and references therein). Following recommendations of Derolez and
214 collaborators, we partitioned lagoons over a multidimensional gradient running a principal
215 component analysis over all environmental variables. The three first principal components of
216 this analysis explained 52.7%, 17.8% and 12.6% of the total variance (83.1% in total; ESM1),
217 highlighting three fundamental descriptors. Principal component 1 (PC1) loaded strongly and
218 positively on total nitrogen, total phosphorus, chlorophyll *a*, phosphates and turbidity,
219 representing increasing organic matter inputs and primary productivity with increasing values
220 of PC1 and highlighting eutrophication (Derolez et al., 2020). PC2 loaded strongly and
221 positively on lagoon morphology (increasing values of PC2 reflecting increasing lagoon
222 surface and depth), whereas increasing values of PC3 reflected increasing concentrations of
223 dissolved inorganic nitrogen and decreasing salinity in the water column (ESM1). Each
224 lagoon was then characterized by its centroid value on each of these three axes that have been
225 considered in the following analyses to describe the environment.

226

227 **Fish sampling**

228 Seabream were sampled from the same ten coastal lagoons in autumn 2017. Fish were
229 sampled just before their annual winter migration from the lagoons. Individuals were fished
230 using traditional Mediterranean fishing gear (“capetchades”) from October 10th to 30th 2017.
231 This fishing method uses a thin mesh to capture and sample live fish. The device was
232 deployed in the evening; fish entered during the night and were collected early the next
233 morning. We captured 100 individuals, focusing on the smallest fish, to insure all were young
234 individuals and incidentally males (as seabreams are proteandrous hermaphrodites). Fish were
235 kept in oxygenated lagoon-water tanks until sacrificed by immersion in ice-cooled lagoon-
236 water tanks and a blood sample collected within 1 hour of capture. We collected 0.5 mL of
237 blood from the caudal vein using a 25G heparinised syringe. After centrifugation (3500g for

238 10 min) plasma was separated and frozen in a dry shipper containing liquid nitrogen (~-
239 190°C, ©CX100 Worthington Industries) and then kept frozen at -80°C in the lab until
240 analysed. Individuals were weighed to the nearest \pm 1g and their total length measured to the
241 nearest \pm 1mm using a metal ruler (Table 1). Fish were kept on crushed ice for transport to the
242 lab, then frozen at -20°C until analyses. Four weeks later, all individuals were unfrozen and
243 we collected the scales over the entire right flank of the fish flank for stress hormone
244 (cortisol) content measures, and 20 to 30 scales below the pectoral fin on the opposite flank to
245 confirm individual age from scale growth annuli. Three pieces of dorsal epaxial muscle (white
246 muscle) were saved and kept frozen at -80°C for contaminant, lipid and protein content
247 analyses. Muscle samples were collected using ceramic tools and rinsed with ethanol 99% and
248 double-deionized water (MilliQ) between each individual to avoid any contamination. Muscle
249 weight was measured before and after lyophilisation to account for individual hydration level.
250 Measurements on muscle (contaminant, lipid, FA) are reported relative to muscle dry weight.
251

252 *Individual age and growth rate*

253 Individual age (in years) was determined by counting annual increments on five to seven
254 scales immersed in aqueous solution using transmitted light, as routinely done in seabream
255 (for details see Mahé et al., 2009). We estimated individual growth rates by dividing
256 individual total length by age. As growth rate drastically decreases after the first year and to
257 account for variation in growth rate between ages, we standardized growth rate by age class
258 (1, 2, 3). Descriptive statistics of total length, mass, age estimated by scale reading, and
259 growth rate centred by age are summarized for each lagoon Table 1.

260

261 **Body composition**

262 *Lipid content*

263 Lipids were extracted from fish muscle following Folch et al. (1957) and lipid classes
264 separated and quantified using an Iatroscan MK-VI (Iatron Laboratories, Inc.) following
265 Parrish (1999) (for details; see Sardenne et al., 2019). Lipids were broadly classified into
266 reserve (e.g. fat stores) or structural (e.g. membrane phospholipids) lipids. Reserve lipids are
267 constituted of triacylglycerols (TAG), diacylglycerols (DAG as precursors of TAG) and free
268 fatty acids (FFA). In contrast, structural lipids include phospholipids (PL) and sterols (ST).
269 Finally, acetone-mobile polar lipids (AMPL), and alcohols (ALC) are eluted at the same time
270 but present in relative low proportions (Tocher, 2003). PL represented the predominant lipid
271 (ca. 87%; 45 to 98% of total lipids, TL) in our samples, whereas TAG concentration showed
272 important inter-individual variation (1 to 51 % of TL). FFA, ALC, DAG and AMPL were
273 very low (<0.05%) or undetected, while ST were always detected at low level (< 2% of TL,
274 see ESM2). Low FFA proportion indicated that lipid integrity was conserved during sample
275 storage (Parrish, 1999).

276

277 *Muscle Protein content and choice of body composition indices*

278 Protein content was assessed using a piece of epaxial muscle after lyophilization and grinding
279 (ball mill, MM400, Retsch GmbH, Germany). Proteins were extracted from 10 mg of dry
280 muscle powder by immersion and incubation in a 1.5-mL solution of a 10% SDS (Sigma
281 Aldrich), 1.5% Protease inhibitor Cocktail (cOmplete, Sigma Aldrich, France), miliQ water
282 solution (lysis solution adapted from Campus et al. 2010). Cycles of successive 15-min
283 ultrasonic baths (300 Ultrasonik, Ney Company, USA) and 3-min vortex were repeated four
284 times to allow total dissolution of the sample. Extracts were clarified by a 10-min
285 centrifugation at 3000g at 4°C (Ericsson and Nistér, 2011). Muscle protein content in 25 µL
286 of the collected fraction was quantified using a Bicinchoninic Acid method (BCA, Pierce,

287 Thermo Fisher Scientific, France). Intra- and inter-plate protein variations (based on the same
288 sample repeated over plates) were 7.62% and 16.7%, respectively.

289 As body composition indices, we used (1) TAG and TAG/PL as indicators of
290 individual endogenous energetic reserves (fish reserve); and (2) PL, protein content, and
291 individual growth rate as indicators of individual investment into structure components (fish
292 structure) (Anedda et al., 2013; Queiros et al., 2019).

293

294 **Trophic markers and Fatty Acid ratio indicators**

295 *Fatty acid identification*

296 The polar fraction of lipids, mainly PL, plays an important role in cell structure and shows a
297 high degree of plasticity in response to changes in abiotic environmental features. The neutral
298 fraction plays a role as a resource compartment, destined either for oxidation to provide
299 energy (ATP) or for incorporation into PL. Predominantly made out of TAG and wax esters,
300 its composition is less influenced by changes in abiotic features and therefore better reflects
301 individual diet (reviewed in Dalsgaard et al., 2003). Therefore, only the neutral fraction was
302 considered in this study. Neutral and polar lipids were separated by column chromatography
303 on silica gel and methylated using 12% BF₃-MeOH (see ESM3). Fatty acid methyl esters
304 (FAMES) were analysed in an Agilent 6890 gas chromatograph equipped with an on-column
305 injector and a flame-ionization detector, with hydrogen as a carrier gas. They were identified
306 by their retention times with reference to those of a standard 37-component fatty acid methyl
307 esters (FAME) mix and designated following the formula C:X ω Y where C is the number of
308 carbon atoms, X is the number of double bonds and Y is the position of the first double bond
309 counted from the CH terminal.

310

311 *Choice of diet indicators*

312 In aquatic food webs, FAs are conservatively transferred along trophic levels (Lovern, 1935).
313 Determining the FA composition of fish tissues has been widely used to track individual diet
314 composition (Dalsgaard et al., 2003; Gao et al., 2006), as well as its quality in terms of
315 essential FAs (Izquierdo 2005, Benedito-Palos et al. 2008, ESM3). As the present study aimed
316 at identifying broad-scale diet profiles, we selected nine generic aquatic trophic indicators
317 (either sum or ratio of specific FA) relevant to aquatic systems (Blanchet-Aurigny et al.,
318 2015), as well as Arachidonic acid (ARA), Docosahexaenoic acid (DHA) and
319 eicosapentaenoic acid (EPA) contributions (%) to the neutral lipid fraction (Koussoroplis et
320 al., 2011) (Table 2).

321

322 **Metal contaminant load in fish muscle**

323 Muscle samples were treated with trace mineral grade nitric acid (69% Suprapure, Merck) and
324 33% H₂O₂ (Suprapure, Merck) in Teflon reaction tubes, heated in a microwave digestion
325 system (UltraClave-Microwave Milestone®) for 20 min at 220 °C, and then diluted to 10 mL
326 with double-deionized water (MilliQ). Total mercury (Hg) content was measured using an
327 atomic absorption spectrometer AMA254 Advanced Mercury Analyzer (Leco) (Bouchoucha
328 et al., 2018), without pre-treating or pre-concentrating the samples (wavelength = 253.65 nm,
329 detection limit (DL) = 0.003 µg.g⁻¹). Samples were further analysed using inductively coupled
330 plasma optical emission spectrometry (ICP-OES, ICAP 6500 Duo, Thermo) to quantify levels
331 of aluminium (Al), arsenic (As), boron (B), cadmium (Cd), chrome (Cr), copper (Cu), nickel
332 (Ni), lead (Pb), rubidium (Rb), Selenium (Se) and zinc (Zn) (García-Navarro et al., 2017).
333 The DL for the analysed elements was 0.001 µg.g⁻¹. Each sample was read in duplicate and
334 averaged. Uncertainty remained between 4.12 and 6.43% (Al: 5.38%, As: 4.23%, B: 4.82%,
335 Cd: 4.56, Cr: 4.30%, Cu: 4.12%, Hg: 5.26%, Ni: 4.83%, Pb: 6.14%, Rb: 5.36%, Se: 6.43%

336 and Zn: 5.02%). Based on UNE-EN ISO reference 11885, multi-element calibration standards
337 (SCP Science, in 4% nitric acid) were assembled with different concentrations of inorganic
338 elements.

339

340 **Individual stress response**

341 *Stress hormone: scale cortisol content*

342 We assessed chronic individual stress response levels by measuring scale cortisol levels
343 (adapted from Carbajal et al. 2018), the principal glucocorticoid in teleost. Measuring scale
344 content, after removing external cortisol from mucus, allows assessing glucocorticoid
345 secretion retrospectively over an extended period of time (Aerts et al., 2015; Sadoul and
346 Geffroy, 2019). The scales of an entire flank of each individual were collected, washed and
347 vortexed three times (2.5 min; 96% isopropanol) to remove external sources of cortisol.
348 Residual solvent traces were evaporated under nitrogen flux, samples frozen at -80°C and
349 lyophilised for 12 hours before grinding to a powder (ball mill, MM400, Retsch GmbH,
350 Germany). Cortisol was extracted from ~20 mg of dry scale powder by incubation in 1.5mL
351 of methanol (MeOH) on a 30°C rocking shaker for 18 hr. After centrifugation (9500g, 10
352 min), the supernatant was evaporated under nitrogen flux and reconstituted with 0.2 mL of
353 EIA buffer provided by the cortisol assay kit (Neogen® Corporation Europe, Ayr, UK).
354 Cortisol concentrations were determined in 50 µL of extracted cortisol samples using
355 competitive EIA kits (Neogen® Corporation Europe, Ayr, UK). Samples were run in
356 duplicates and averaged. Intra-plate variation (each sample run in duplicates) was 3.36% and
357 inter-plate variation (2 control samples were run in duplicate over all plates) was 5.67%.

358

359 *Fish oxidative status*

360 We assessed global measures of oxidative damages and antioxidant defences in plasma using
361 the d-ROMs test and OXY Adsorbent tests (Diacron International©, Grosseto, Italy).
362 Oxidative damage was assessed in 8 μL of plasma by measuring hydroperoxides, the main
363 compounds contributing to plasma oxidant activity, expressed as mg H_2O_2 equivalent/dL
364 (Beauvieux et al., 2022). Oxidative defences were evaluated in 5 μL of 1:100 diluted plasma
365 by measuring the ability of plasma to buffer massive oxidation through hydroperoxide acid,
366 expressed in $\mu\text{mol HCl/mL}$ (Beauvieux et al., 2022). All sample measurements were run in
367 duplicate and averaged. Intra-plate variations (each sample run in duplicates) were 2.40% and
368 7.51% for ROMs and OXY, respectively. Inter-plate variations (2 control samples were run in
369 duplicate over all plates) were 10.02% and 7.92% for ROMs and OXY, respectively.

370

371 *Immune functions: Natural haemolytic complement and lysozyme activities*

372 Fish immune systems are precursors and quite primitive relative to that of most vertebrates,
373 mostly relying on non-specific/innate/natural immunity. The complement and lysozyme
374 systems are important mechanisms of the immune system for recognizing and eliminating
375 non-self-substances in fish (Sitjà-Bobadilla et al., 2003; Tort et al., 1998). Plasma activity of
376 the complement pathway was assayed against rabbit red blood cells collected in Alsever's
377 solution (Sigma Aldrich, Lyon, France). SRBC were washed in phenol red-free Hank's buffer
378 (HBSS) containing Mg^{2+} and EGTA and re-suspended at 3% (v/v) in HBSS. 100 μL test
379 serum was serially diluted in HBSS solution to obtain four final serum concentrations ranging
380 from 10% to 0.078% in a round-bottomed 96-well plate. An equal volume (100 μL) of SRBC
381 was added to each well. Following incubation for 90 min at room temperature, samples were
382 centrifuged at 400 g for 5 min at 4°C to remove un-lysed erythrocytes. The relative
383 haemoglobin content of supernatants was assessed by measuring its optical density at 550 nm
384 (©Tecan Infinite 200 microplate reader). The values of maximum (100%) haemolysis were

385 obtained by adding 100 mL of distilled water to 100 mL samples of SRBC and values of
386 minimum (spontaneous) haemolysis obtained from SRBC without serum. The power of
387 haemolysis (%) was estimated as the slope of its lysis curve (Díaz-Rosales et al., 2006). Intra-
388 (each sample run in duplicates) and inter-plate (2 control samples were run in duplicate over
389 all plates) variations were 9.06% and 14.71%, respectively.

390 Lysozyme activity assays were performed by a turbidimetric method using the lysis of
391 *Micrococcus luteus* by plasma samples to determine their enzymatic activity. Serum lysozyme
392 activity was measured according to methods described by Ellis (1990) and previously applied
393 to gilthead seabream (Tort et al., 1998). Briefly, 25 μL of individual serum was mixed with a
394 75 μL *Micrococcus lysodieticus* (Sigma Aldrich, Lyon, France) suspension at 75 $\mu\text{g}\cdot\text{mL}^{-1}$ in
395 0.1 M phosphate citrate buffer, pH 5.8. After rapid mixing, turbidity changes were measured
396 every 60s over 30 min at 415 nm, 28°C (©Tecan Infinite 200 microplate reader). The
397 dilutions of chicken egg white lysozyme (Sigma Aldrich) served as a standard. Lysozyme
398 activity was estimated by comparing individual dynamic turbidity decrease over time
399 compared to that of the standard's and is expressed as Unit/mL serum. Samples were run in
400 duplicate and averaged. Intra-plate (each sample run in duplicates) and inter-plate (2 control
401 samples were run in duplicate over all plates) variations were 15.7% and 5.68%, respectively.

402

403 **Statistics**

404 All statistical analyses were run in the R v3.5.1 (R Development Core Team 2008). We
405 gathered all information required for the present study for 96 out of the 100 sampled fish (age
406 0: N=42, age 1: N=27 & age 2: N=48). For four individuals, one or two assays were lacking
407 due to limited blood material and were removed from the analysis. Altogether we thus
408 considered 96 individuals * 39 variables = 3744 data points (see ESM1 & 6 for more details).

410 We investigated the multi-scale causal pathways by which extrinsic (environmental
411 and anthropogenic) and intrinsic factors affected seabream ecophysiology using Partial Least
412 Squares Path Modelling (PLS-PM) (R package `pls`, Sanchez 2013). PLS-PM allows
413 studying complex multivariate relationships among groups of manifest (observed) and latent
414 (construct) variables (MVs and LVs respectively), testing for direct and indirect effects. These
415 effects are necessarily unidirectional (Sanchez, 2013; Vinzi et al., 2010) and can be used to
416 draw causal inferences in the path diagram (more detailed framework presented in ESM4).
417 LVs are hypothetical constructs that are not measured directly but can be assumed to reflect
418 their underlying MVs. The general construct of this study is detailed in Fig. 1.

419 Using a PLS-PM approach, we defined extrinsic and intrinsic constructs (LVs), built
420 in a formative way (for detail see ESM4), characterizing relevant environmental and
421 physiological pressures affecting seabreams in coastal lagoons. We then explored how
422 individual stress response was related to those morphological and hydrobiological,
423 anthropogenic (contaminant), dietary and intrinsic constructs using an iterative algorithm
424 (Trincherá and Russolillo, 2010), and comparing seven alternative models (Table 3). The 7
425 constructs (LVs) always included: (1) lagoon features (morphological and hydrobiological);
426 and fish (2) diet, (3) contaminant loads, (4) endogenous reserves, (5) structure components,
427 (6) age, and (7) stress response. Each of these latent variables was the combination of one or
428 more MVs (such as lagoon eutrophication, lagoon depth, fish cortisol levels, etc.). Expected
429 directional relationships were based on empirical and theoretical research to draw a
430 comprehensive picture of the complex ecosystem interactions likely affecting seabream/fish
431 stress response in coastal lagoons, and alternatives were assessed for seven different causal
432 models (see ESM5). We tested for potential effects of lagoon features on fish age (Model 2),
433 and tested if the LV fish age was best predicted by structural size, age in years or both (Model

434 1, 3 & 4). Models 1, 5 & 6, differed in terms of directional relationships (e.g. fish reserve
435 affected fish structure, the opposite, or no relation was specified between those LVs; Table 3).
436 We computed a final model including all of the interactions retained in previous models
437 (Model 7). For each model, we computed and compared the overall variance explained by the
438 path model (goodness of fit, GoF) (Table 3, ESM5). The model displaying the highest GoF
439 was retained and discussed in the manuscript.

440 First, we consider the model from an integrative perspective. We consider which
441 resources individuals might extract from the environment and how they might distribute those
442 to different functions, ultimately asking how individuals deal with environmental variations in
443 terms of stress management. We therefore describe causal relationships likely linking a given
444 LV to other independent LVs in our model. Second, we discuss the indirect effects affecting
445 individual stress response. The overall path model (PLS-PM; Fig. 3) represents: (1) the
446 manifest models including all correlations between MVs (*e.g.* lagoon eutrophication, depth,
447 area, dissolved inorganic nitrogen and salinity) and their respective LVs (*e.g.* lagoon
448 features); and (2) the structural model in which all causal relationships between our LVs
449 (lagoon features, fish age, fish diet, fish reserve, fish structure, fish contaminant load, fish
450 stress) are determined.

451

452 **RESULTS**

453 **Model Comparisons**

454 The seven models displayed similar global explanatory performances (GoF) ranging from
455 27.3% to 32.6% (Table 3). Regardless of the model considered the relationships between
456 connected latent variables (LVs) that were significant remained identical in terms of sign

457 (positive or negative; ESM5) and only differed slightly in terms of strengths. Model 7
458 displayed the highest GoF (32.6 %) and therefore was selected for further investigation.

459

460 **Global model assessment: overall variance explained by the path model 7**

461 *Manifest model variance*

462 The variance of the manifest variables (MVs) reflected by the latent variables (LVs) is given
463 by the block communality coefficients (Table 5). For instance, the LV “lagoon features”
464 reflected 44% of the variance in its three MVs, *i.e.* the three principal components of the PCA
465 representing eutrophication, lagoon morphology (depth and area), and dissolved inorganic
466 nitrogen (DIN) and salinity variables (Table 5). The LV “fish age” included the age and size
467 of fish and accounted for 94% of the variation in age and size. Other LVs shared a lesser
468 proportion of variance with their underlying MVs, ranging from 12 to 36%. Table 5
469 summarizes all explained variance from LVs and Fig. 3 describes how they related to the
470 underlying (measured) MVs. First, the LV “lagoon features” was positively related to
471 eutrophication ($r = 0.91$), lagoon depth and area ($r = 0.60$) and the third PCA axis
472 encompassing increasing DIN and decreasing salinity ($r = 0.36$). Second, the LV “fish diet”
473 assessed by measurement and composition of free fatty acid, was negatively related to
474 dinoflagellates 2 ($r = -0.51$), diatoms ($r = -0.49$), terrigenous inputs ($r = -0.33$), bacteria ($r = -$
475 0.18), detrital sources ($r = -0.65$), and arachidonic acid (ARA) ($r = -0.34$) and
476 eicosapentaenoic acid (EPA) ($r = -0.69$), while positively related to carnivorous ($r = 0.78$) and
477 copepods ($r = 0.15$) inputs. DHA ($r = 0.10$), dinoflagellates 1 ($r = -0.06$), and non-methylene
478 interrupted (NMI) ($r = -0.06$) contributions to the LV “fish diet” remained small ($r \leq 0.10$).
479 Third, the LV “fish reserve” corresponded to an increase in TAG ($r = 0.35$) and TAG/PL ratio
480 ($r = 0.56$), both being proxies of fish body condition. Fourth, the LV “fish structure”

481 positively correlated to growth rate ($r = 0.52$) and negatively correlated ($r = -0.90$) with the
482 amount of phospholipid present in the neutral FA stored. Muscle protein concentration only
483 marginally contributed to the LV “Fish structure” ($r = 0.07$). Fifth, the LV “fish contaminant”
484 load positively correlated to metal concentrations of Al ($r = 0.40$), As ($r = 0.14$), B ($r = 0.50$),
485 Cu ($r = 0.57$), Rb ($r = 0.35$), Zn ($r = 0.46$), Hg ($r = 0.20$) and negatively correlated to
486 concentrations of Se ($r = -0.51$) and Ni ($r = -0.11$). Cd, Cr, and Pb contributions were
487 marginal ($r \leq 0.10$). Finally, the LV “Fish stress” highly and positively correlated to cortisol
488 ($r = 0.71$), oxidative damage (ROM, $r = 0.67$), complement ($r = 0.29$), and antioxidant
489 defences (OXY $r = 0.17$) levels. In contrast, it was negatively related to lysozyme activity ($r =$
490 -0.24) (see Fig. 3 and ESM6).

491

492 ***Structural model variance***

493 Within the structural model, the amount of variance of a given LV (*e.g.* fish contaminant
494 load) that is explained by all other LVs (lagoon features, etc.) in the path model is presented
495 Table 5 (R^2 values). The amount of explained variance in LVs “fish structure” (55%), “fish
496 contaminant” (63%) and “fish stress” (54%) was high. In contrast, the amount of explained
497 variation for LV “fish reserve” was relatively low (16 %) and the amount of variation in LV
498 “fish diet” explained by other LVs (fish age and lagoon features) was intermediate (28 %).

499 *Direct effects:* Starting from the top of our constructs, individual diet composition was
500 affected by individual age and environmental lagoon features encountered during the summer
501 (Fig. 3; Table 6). The greater lagoon eutrophication, depth, area and dissolved inorganic
502 nitrogen content, the more fish diet shifted from planktivorous, terrigenous, detrital, and EPA
503 sources to carnivorous ones. The same relationship was found as fish age increased.
504 Consequently, when fish shifted to a more carnivorous diet, structural lipid concentrations

505 decreased but growth increased (LV “fish structure”). Such diets had a positive effect on fish
506 energy stores (reserve variable), reflected by increased TAG and TAG/PL ratio. Age showed
507 opposite trends on fish reserve and fish structure: increasing age was associated with
508 increasing structural lipids (although limited growth) but decreasing energy stores. We found
509 no substantial effect of the LV “lagoon feature” on fish reserves or fish structure. Notably,
510 fish structure was strongly affected by fish reserve with higher reserves leading to higher
511 growth.

512 Fish age was also negatively associated with muscle contaminant concentrations,
513 whereas fish reserve, structure and diet had non-significant effects on contaminant
514 concentrations. Most importantly, fish contaminant concentrations had the highest direct
515 positive (+0.38) impact on fish stress response. Increasing lagoon eutrophication, depth, area,
516 dissolved inorganic nitrogen and decreasing salinity (LV “lagoon feature”) were associated
517 with increased fish stress response (+0.25), whereas stress response largely decreased with
518 increasing fish age (-0.41). Finally, the direct effects of individual diet and energy stores (LV
519 “Fish reserve”) on individual stress response was non-significant (Fig. 3, Table 6).

520

521 *Indirect effects:* The PLS-PM framework further allowed assessing indirect effects by
522 multiplying coefficients along the path. Focusing on total effects affecting fish stress
523 response, positive direct effects of lagoon features (+0.25) on individual stress response was
524 counteracted by negative indirect effects through muscle contaminant loads (total effect of
525 lagoon features on contaminant load * total effect of contaminant on stress response = $-0.77 * 0.38 = -0.29$). The later was slightly balanced out by indirect effect through fish diet ($0.57 * 0.20 = 0.11$), fish reserve ($0.22 * 0.10 = 0.02$) and fish structure ($0.28 * 0.17 = 0.05$) (Figure 4
528 and ESM7). As a result, the overall effect of lagoon features on fish stress response was low
529 (+0.11). In contrast, direct and indirect age effects on fish stress response were cumulative

530 leading to the highest negative coefficient (-0.61): increasing age was associated with
531 decreasing stress loads through direct and indirect effects. The indirect effects of fish diet,
532 reserve and structure on fish stress response were marginal (<0.06). A summary of direct and
533 indirect effects is given Figure 4 (for a full description of the other LVs relationships see
534 ESM7).

535

536 **DISCUSSION**

537 Using a multifactorial PLS-PM analysis, we identified key direct and indirect contributions of
538 extrinsic (lagoon features, fish diet, fish contaminant loads) and intrinsic (fish reserves, fish
539 structure components, fish age) factors affecting fish stress response in coastal lagoons.
540 Understanding how individuals deal with multiple ecological pressures using stress response
541 as a proxy is a first step to better understand the constraints that apply to population dynamics
542 (Brosset et al., 2021; McKenzie et al., 2016; Saraux et al., 2019). Although a larger sample
543 size might have improved the overall statistical power, the PLS path model explained 33% of
544 the total variance of the 46 variables accounted in this study, reflecting ten Mediterranean
545 lagoon systems, a substantial amount considering that the work was performed at the scale of
546 multiple ecosystems. More importantly, 54% of the variance of the LV “fish stress” was
547 explained by the dependent LVs “lagoon features”, “fish age”, “fish diet”, “fish reserve”,
548 “fish structure”, and “fish contaminant” included in the model.

549

550

551 *Extrinsic and intrinsic factors affecting fish metal contaminant loads*

552 Lagoon features had an important negative effect on fish contaminant loads. When the
553 LV “lagoon features” increased, eutrophication (inorganic matter content and primary

554 production) increased, while salinity decreased. This is consistent with the fact that both the
555 toxicity of metal contaminant to fish and their bioavailability in water are known to vary with
556 water physicochemical properties, including pH, alkalinity, suspended solids, organic
557 compound content, and hardness (Di Giulio and Meyer, 2008). Increasing organic matter
558 concentration reduces metal accumulation to aquatic organisms by reducing the amount of
559 free cationic metal by chelation (Richards et al., 2001; Schwartz et al., 2004). In rainbow
560 trout, increased dissolved organic matter concentration has been associated to increased
561 survival when faced with lethal Pb and Cu metal concentrations allowing reduced binding to
562 gills (Richards et al., 2001; Schwartz et al., 2004). In contrast, while increasing salinity has
563 been shown to reduce metal contaminant accessibility in bivalves and some teleost fish
564 (Somero et al. 1977; Lee et al. 1998; Ifremer 2009), its bioavailability in the water and
565 sediments tend to increase (Zhao et al., 2013). Our results suggest that for seabream the
566 opposite effect of eutrophication and salinity on contaminant concentration in the fish could
567 be due either to differences in gill osmoregulation processes in seabream compared to other
568 species tested, or non-exclusively, to confounding effects of decreasing salinity and increasing
569 organic matter concentration affecting contaminant loads. Indeed, the strong negative effect (-
570 0.65) of the LV “lagoon feature” on fish contaminant load could be due to a stronger effect of
571 increasing organic matter than decreasing salinity (higher loading and weight of
572 Eutrophication of Lagoon features compared to Salinity; ESM6).

573 We also found that Gilthead seabream displayed a general pattern of metal
574 contaminant bio-diminution with age. The PLS-PM showed that contaminant load decreased
575 markedly with age (direct effect of fish age on contaminant load; -0.55, Fig. 3, Table 6).
576 When focusing on individual metal concentrations and their respective relationships with the
577 total length of individual (see ESM8), we found that aluminium (Al), boron (B), copper (Cu),
578 nickel (Ni), rubidium (Rb), selenium (Se) and zinc (Zn) concentrations were all negatively

579 related to total length. This suggests that dilution of those metals occurred with age-related
580 changes in diet shift and size (Lee et al., 1998) and is consistent with similar relationships that
581 have been described in trophic webs (cephalopods, coelenterates, crustaceans, echinoderm,
582 fish, and gastropods) for cadmium (Cd) and lead (Pb) in freshwater and marine ecosystems
583 (Asante et al., 2008). In contrast, arsenic (As) and mercury (Hg) were the only two elements
584 to bioaccumulate and/or biomagnify in seabream (reflected by positive correlations of their
585 concentration with size). Cd, chromium (Cr) and Pb showed no relationship with total length
586 (ESM8). This absence of relationships suggests that these metals were neither biomagnified
587 nor biodiminished during ontogenetic development or through the food web. Although these
588 relationships deserve to be considered with cautious, indeed the relatively low overlap of age
589 classes between each lagoon do not allow to exhaustively disentangle age and environmental
590 effects, similar bio-accumulation and dilution are common in marine organisms and food
591 webs, including seabreams (Chouvelon et al., 2014; Cresson et al., 2015; Harmelin-Vivien et
592 al., 2012).

593

594 ***Synergetic effects and consequences for individual body composition and growth***

595 Lagoon features had an overall (direct + indirect) positive effect on fish reserve (+0.22) and
596 fish structure (+0.28). Increasing relative eutrophication (without decreasing dissolved O₂
597 concentration ESM1B) was positively linked to fish growth rates and the quantity of
598 endogenous reserves individuals accumulated during the summer. This concurs with previous
599 findings that seabream growth and body condition between and within lagoons generally
600 increase with increasing organic matter inputs (Brehmer et al., 2013; Escalas et al., 2015;
601 Isnard et al., 2015). These two effects were largely captured indirectly in our model (direct
602 effects non-significant; coefficients < 0.05; Table 6) by dietary shifts from planktivorous,
603 terrigenous, detritic, and EPA-based diets to carnivorous diets when eutrophication increases.

604 Further, lagoon features and fish age appear to act synergistically on fish diet: as individuals
605 age and grow, they progressively switch to bigger prey, which at juvenile stage lead to the
606 developing of a more carnivorous diet toward molluscs and fish larvae (Escalas et al., 2021;
607 Rosecchi, 1987).

608

609

610 *Integrating multiple factors in our understanding of fish stress response and health*

611 We found that fish age (age in years and size) displayed the strongest direct effect on
612 stress response (-0.41). Older individual are thought to have greater experience since they
613 have logically faced and survived several challenges as they age. As they age, individuals that
614 are exposed to stressors might also develop mitigating strategies allowing them to deal with a
615 variety of challenges and acclimate resulting in decreasing stress responses (Moreira et al.,
616 2019). Moreover, fish that survive longer may be of better intrinsic quality (biased selection),
617 leading to individuals displaying lower maintenance costs in the sampled population (Birnie-
618 Gauvin et al., 2017; Wilson and Nussey, 2010). These non-exclusive hypotheses may explain
619 the lower stress response of older (max. 3 years old) individuals.

620

621 Interestingly, the effect of several lagoon features on stress response was independent of the
622 other studied LVs, *i.e.* not captured as indirect effects but as direct effects (+0.25). In other
623 words, increasing eutrophication, lagoon morphology (depth and surface), inorganic nitrogen,
624 and decreasing salinity directly increased individual stress response. This may be due to direct
625 effects on individual physiology, such as individual osmoregulation processes, that can be
626 energetically stressful when salinity decreases and eutrophication increases (Bodinier et al.,
627 2010; Cuesta et al., 2005). An alternative may be that these effects are captured indirectly,
628 through other unmeasured lagoon ecosystem features known to increase stress and decrease

629 immune functions, for instance predation risk (Werner et al., 1983) or competition (Goldan et
630 al., 2008; Salati et al., 2016), which deserve further investigation.

631

632 Fish structure (decreasing PL and increasing growth rate) had a non-significant effect (+0.18,
633 $p=0.089$) on fish stress response (increase in cortisol level, oxidative damages, anti-oxidant
634 defences and complement activity, and a decrease in lysozyme activity). Nonetheless, from a
635 life-history perspective at the organism level, it is assumed that the greater the investment in
636 growth, the lower the investment in prevention or repair of molecular damage (Cichon, 1997;
637 Stearns, 1989). Pushing physiological machinery to grow faster may lead to metabolically
638 induced damage (Metcalf and Alonso-Alvarez, 2010; Monaghan et al., 2009). Studies in
639 several taxa indicate that the trade-off underlying accelerated growth early in life comes at an
640 oxidative cost (Merry, 1995). Long-term consequences may be offset by subsequent impaired
641 performance, including decreased immunological competence, energy reserves and longevity
642 (Inness and Metcalfe, 2008; Rollo, 2002), or reduced investment in protein maintenance
643 (Morgan et al., 2000). This is notably the case for animals having experienced a period of
644 slow growth followed by rapid compensation (compensatory growth), relative to those having
645 grown steadily throughout (Metcalf and Monaghan, 2001). However, there are many
646 ecological advantages to attaining a large body size as fast as possible. Reaching maturation
647 rapidly may be a successful strategy in terms of reproductive success or competition for food
648 resources (Lee et al., 2012; Scharf et al., 2000). Larger sizes may also be associated with
649 reduced risks to predation (Sogard, 1997). Such long-term benefits may outweigh the short-
650 term stress costs of investing in structural tissues and growth.

651

652 Most importantly, our analysis shows that when considering several component of the
653 costal lagoon ecosystem and physiological trait of the gilthead seabream, its stress response

654 was mostly affected by muscle inorganic contaminant content. Over the past decade, similar
655 causal relationships have been shown by studies on contaminant loads under controlled
656 laboratory conditions. Exposure to heavy metals is usually associated with increased cortisol
657 and oxidative stress levels (Sevcikova et al., 2011) and lower immune function (Cerezuela et
658 al., 2016; Guardiola et al., 2016, 2015). In small amounts, metal elements such as As, Cu, Cr,
659 Ni, and Zn are essential for immune and oxidative defence functions, whereas they impair
660 these same functions in excess (Sevcikova et al., 2011). For instance, Zn or Cu deficiencies
661 lead to deficiencies in immune and antioxidant systems and to the development of life
662 threatening infections (Rink and Kirchner, 2000). In contrast, at high doses, those same
663 elements have inhibiting effects on the immune system (Rink and Kirchner, 2000) or increase
664 oxidative stress (Noh and Koh, 2000; Valko et al., 2005). Because of their deleterious impacts
665 at low concentration on organism physiology, As, Cd, Hg and Pb are the most commonly
666 (often individually) studied metal contaminants. Here, we considered a wider array of
667 contaminant, considering cocktail effects in interaction with other environmental features
668 rather than single contaminant effects. It is remarkable that individual contaminant loadings
669 on the LV “contaminant load” were relatively low for these widely studied metal contaminant
670 (As +0.14, Cd +0.02, Hg +0.20 and Pb +0.10). Rather, it appeared that interactions between B
671 (+0.50), Cu (+0.57), Rb (+0.35), Zn (+0.46), Al (+0.40) and a decrease in Se (-0.56) were
672 critical in explaining individual stress response in seabream. It is important to understand that
673 the physiological action of these two different groups of contaminant in causing oxidative
674 stress are fundamentally different (Valko et al., 2005). Metals without redox potential, such as
675 As, Cd, and Hg, cause oxidative stress by impairing antioxidant defences, especially those
676 involving thiol-containing antioxidants and enzymes (Stohs and Bagchi, 1995). In contrast,
677 redox active metals such as Fe, Cu and Al participate in the formation of reactive oxygen
678 species (*e.g.* Fe and Cu catalyse the formation of reactive hydroxyl radicals ($\bullet\text{OH}$) by Fenton

679 reaction) (Gaetke and Chow, 2003; Liaquat et al., 2019). Elements such as dietary Se have
680 been shown to protect against lipid peroxidation and Cu (Kadiiska and Mason, 2002) and Cd
681 toxicity (Ulusu et al., 2003). The above highlights the complexity of interactions between
682 contaminant, and the importance of integrated approaches to evaluate cocktail effects.

683 Measuring cortisol in the scales of the fish, enabled us to assess a proxy of the total
684 amount of corticoid hormone secreted through the life of the individual (Goikoetxea et al.,
685 2021; Harris, 2020; Sadoul and Geffroy, 2019). Cortisol level accumulated in the scales was
686 highly loaded on the LV “stress response”, therefore its regulation tended to positively relate
687 to an increasing long term stress response. Circulating glucocorticoid hormone is commonly
688 used as a marker of individual stress response as they mediate a cascade of physiological
689 (increased metabolism) and behavioural response to stress (Munck et al. 1984, Johnson et al.
690 1992) and has been widely employed to evaluate several stressor effects in fish, from disease
691 to chemical contaminants (Zimmer et al., 2011). Although under acute exposure its
692 concentration tend to increase and lead to rapid mitigating response toward a stressor and
693 protection against toxic effects (Bury et al., 1998; Laflamme et al., 2000), long term intense
694 stress event may induce a decrease of glucocorticoid secretion and a decline in the
695 hypothalamic–pituitary–adrenal response to stress with subsequent consequences on
696 immunity and reproduction (Hontela et al., 1997; for a meta-analysis see Rohonczy et al.,
697 2021). In the present study, the stress response of the fish was highly related to increasing
698 contaminant load. It suggests that long term exposure led to constant increase in cortisol
699 secretion without impairing their ability to mount an efficient HPA regulation. Although
700 critical stress responses do not seem to be reached, contrary to some acute responses observed
701 in experimental approaches (Hontela et al., 1997; Hontela and Fortin, 1995), it still rises
702 questions on the long term consequences of such maintained high stress response on future
703 reproduction and metabolism trade-offs.

704 Over the last decade, contaminant monitoring programs for coastal ecosystems were
705 developed for the Mediterranean Sea to provide scientific knowledge to assess ecosystem
706 health and sustainability (Bonito et al., 2016; Naccari et al., 2015; Tomasello et al., 2012).
707 However, mainly due to European legislation, most studies on fish species have focused on
708 As, Cd, Hg and Pb contamination or have compared contaminant loads for different species at
709 different locations (Cresson et al., 2016; Mille et al., 2018; see Chouvelon et al., 2017 for a
710 multi-population approach for tuna), although few studies have focused on the consequences
711 on individual health in nature. Yet, as monitoring of organic contaminants such as POPs
712 (HAPs, PFOS, PFAS, DDT, Lindane...) become more common in marine environments, it
713 urges further research that would certainly bring new insights on the impact of changing
714 lagoon environment on fish health (Munaron et al., 2023).

715

716 *Conclusion*

717 Using the Gilthead seabream and Mediterranean lagoons as a model, our study emphasized
718 the importance of integrated approaches to identify direct and indirect contributions of
719 multiple environmental factors affecting individual stress response in coastal ecosystems.
720 Abiotic and biotic features of the environment have complex direct and indirect, synergetic or
721 antagonistic, interactions with individual intrinsic features. A proper evaluation of these
722 effects on stress response and health requires multi-factorial frameworks and may benefit
723 from further inclusion into energetic models (such as the allostasis or reactive scope models)
724 aiming to understand the tipping-point at which physiological systems are pushed beyond
725 their capacity to cope with stressors in the environment leading to pathology and disease
726 (McEwen & Wingfield 2003; Romero et al. 2009). The challenge ahead lies in extending such
727 approaches from the individual to population scale.

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751

752 **AUTHORS CONTRIBUTION**

753 QS, CS, VAV and JB designed the experiment and collected the data. QS, LM, AB and LL
754 developed the methods and did most of the laboratory analyses. DR measured metal
755 contaminant concentrations in all seabream muscles. VD and DM managed the long term
756 environmental data collection (OBSLAG-WFD), provided environmental datasets, and
757 contributed to the interpretation of lagoons features. CQ and FP identified and quantified fatty
758 acid. CWM provided logistical and financial support in the realisation of the study. QS did the
759 statistical analyses and wrote the manuscript. CS, VAV, CWM, AB and JB commented on the
760 paper. All authors agreed on the final version.

761

762 COMPLIANCE WITH ETHICAL STANDARDS

763 All experiments were approved by the national ethic comitee (APAFIS#8945-201
764 7021612469374 v4 & APAFIS#10759-2017072512353814 v3) and realized in compliance
765 with French and European Law.

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768 REFERENCES

769

- 770 Aerts, J., Metz, J.R., Ampe, B., Decostere, A., Flik, G., De Saeger, S., 2015. Scales Tell a Story on the Stress
771 History of Fish. PLoS One 10, e0123411. <https://doi.org/10.1371/journal.pone.0123411>
- 772 Anedda, R., Piga, C., Santercole, V., Spada, S., Bonaglini, E., Cappuccinelli, R., Mulas, G., Roggio, T., Uzzau,
773 S., 2013. Multidisciplinary analytical investigation of phospholipids and triglycerides in offshore farmed
774 gilthead sea bream *Sparus aurata* fed commercial diets. Food Chem. 138, 1135–1144.
775 <https://doi.org/10.1016/j.foodchem.2012.11.098>
- 776 Arts, M.T., Kohler, C.C., 2009. Health and condition in fish: the influence of lipids on membrane competency
777 and immune response, in: Lipids in Aquatic Ecosystems. Springer New York, New York, NY, pp. 237–
778 256. https://doi.org/10.1007/978-0-387-89366-2_10
- 779 Asante, K.A., Agusa, T., Mochizuki, H., Ramu, K., Inoue, S., Kubodera, T., Takahashi, S., Subramanian, A.,
780 Tanabe, S., 2008. Trace elements and stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in shallow and deep-water
781 organisms from the East China Sea. Environ. Pollut. 156, 862–873.
782 <https://doi.org/10.1016/j.envpol.2008.05.020>
- 783 Auel, H., Harjes, M., Rocha, R., Stübing, D., Hagen, W., 2002. Lipid biomarkers indicate different ecological
784 niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellula*.
785 Polar Biol. 25, 374–383. <https://doi.org/10.1007/s00300-001-0354-7>

786 Barcellos, L.J.G., Kreutz, L.C., Koakoski, G., Oliveira, T.A., da Rosa, J.G.S., Fagundes, M., 2012. Fish age,
787 instead of weight and size, as a determining factor for time course differences in cortisol response to
788 stress. *Physiol. Behav.* 107, 397–400. <https://doi.org/10.1016/j.physbeh.2012.09.008>

789 Beauvieux, A., Queiros, Q., Metral, L., Dutto, G., Gasset, E., Criscuolo, F., Fromentin, J., Saraux, C., Schull, Q.,
790 2022. Energy allocation trade-offs between life-history traits in the Mediterranean sardine: an
791 ecophysiological approach. *Mar. Ecol. Prog. Ser.* 701, 99–118. <https://doi.org/10.3354/meps14183>

792 Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: Current status and future opportunities.
793 *Aquaculture* 218, 491–499. [https://doi.org/10.1016/S0044-8486\(02\)00370-8](https://doi.org/10.1016/S0044-8486(02)00370-8)

794 Bell, M., Sargent, J., 1996. Lipid nutrition and fish recruitment. *Mar. Ecol. Prog. Ser.* 134, 315–316.
795 <https://doi.org/10.3354/meps134315>

796 Bell, M. V., Batty, R.S., Dick, J.R., Fretwell, K., Navarro, J.C., Sargent, J.R., 1995. Dietary deficiency of
797 docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.).
798 *Lipids* 30, 443–449. <https://doi.org/10.1007/BF02536303>

799 Benedito-Palos, L., Navarro, J.C., Sitjà-Bobadilla, A., Gordon Bell, J., Kaushik, S., Pérez-Sánchez, J., 2008.
800 High levels of vegetable oils in plant protein-rich diets fed to gilthead sea bream (*Sparus aurata*): Growth
801 performance, muscle fatty acid profiles and histological alterations of target tissues. *Br. J. Nutr.* 100, 992–
802 1003. <https://doi.org/10.1017/S0007114508966071>

803 Benhamed, S., Guardiola, F.A., Martínez, S., Martínez-Sánchez, M.J., Pérez-Sirvent, C., Mars, M., Esteban,
804 M.A., 2016. Exposure of the gilthead seabream (*Sparus aurata*) to sediments contaminated with heavy
805 metals down-regulates the gene expression of stress biomarkers. *Toxicol. Reports* 3, 364–372.
806 <https://doi.org/10.1016/j.toxrep.2016.02.006>

807 Biandolino, F., Prato, E., Caroppo, C., 2008. Preliminary investigation on the phytoplankton contribution to the
808 mussel diet on the basis of fatty acids analysis. *J. Mar. Biol. Assoc. United Kingdom* 88, 1009–1017.
809 <https://doi.org/10.1017/S0025315408001598>

810 Birnie-Gauvin, K., Costantini, D., Cooke, S.J., Willmore, W.G., 2017. A comparative and evolutionary approach
811 to oxidative stress in fish: A review. *Fish.* 18, 928–942. <https://doi.org/10.1111/faf.12215>

812 Blanchet-Aurigny, A., Dubois, S.F., Quéré, C., Guillou, M., Pernet, F., 2015. Trophic niche of two co-occurring
813 ophiuroid species in impacted coastal systems, derived from fatty acid and stable isotope analyses. *Mar.*
814 *Ecol. Prog. Ser.* 525, 127–141. <https://doi.org/10.3354/meps11169>

815 Bodinier, C., Sucré, E., Lecurieux-Belfond, L., Blondeau-Bidet, E., Charmantier, G., 2010. Ontogeny of
816 osmoregulation and salinity tolerance in the gilthead sea bream *Sparus aurata*. *Comp. Biochem. Physiol.*
817 *A. Mol. Integr. Physiol.* 157, 220–8. <https://doi.org/10.1016/j.cbpa.2010.06.185>

818 Bœuf, G., Payan, P., 2001. How should salinity influence fish growth? *Comp. Biochem. Physiol. Part C Toxicol.*
819 *Pharmacol.* 130, 411–423. [https://doi.org/10.1016/S1532-0456\(01\)00268-X](https://doi.org/10.1016/S1532-0456(01)00268-X)

820 Bonito, L.T., Hamdoun, A., Sandin, S.A., 2016. Evaluation of the global impacts of mitigation on persistent,
821 bioaccumulative and toxic pollutants in marine fish. *PeerJ* 4, e1573. <https://doi.org/10.7717/peerj.1573>

822 Boonstra, R., 2013. Reality as the leading cause of stress: Rethinking the impact of chronic stress in nature.
823 *Funct. Ecol.* 27, 11–23. <https://doi.org/10.1111/1365-2435.12008>

824 Boonstra, R., Barker, J.M., Castillo, J., Fletcher, Q.E., 2007. The role of the stress axis in life-history
825 adaptations. *Rodent Soc. An Ecol. Evol. Perspect.* 139–149.

826 Bouchoucha, M., Brach-Papa, C., Gonzalez, J.-L., Lenfant, P., Darnaude, A.M., 2018. Growth, condition and
827 metal concentration in juveniles of two *Diplodus* species in ports. *Mar. Pollut. Bull.* 126, 31–42.
828 <https://doi.org/10.1016/j.marpolbul.2017.10.086>

829 Bouchoucha M., Derolez V., Munaron D., Gonzalez J.L., Cimiterra N., Tomasino C. 2019. Directive Cadre sur
830 l'Eau. Bassin Rhône Méditerranée Corse - Année 2018 . RST.ODE/UL/LER-PAC/19-14.

831 Brehmer, P., Laugier, T., Kantoussan, J., Galgani, F., Mouillot, D., 2013. Does coastal lagoon habitat quality
832 affect fish growth rate and their recruitment? Insights from fishing and acoustic surveys. *Estuar. Coast.*
833 *Shelf Sci.* 126, 1–6. <https://doi.org/10.1016/j.ecss.2013.03.011>

834 Brosset, P., Cooke, S.J., Schull, Q., Trenkel, V.M., Soudant, P., Lebigre, C., 2021. Physiological biomarkers and
835 fisheries management. *Rev. Fish Biol. Fish.* 31, 797–819. <https://doi.org/10.1007/s11160-021-09677-5>

836 Bryndum-Buchholz, A., Tittensor, D.P., Blanchard, J.L., Cheung, W.W.L., Coll, M., Galbraith, E.D., Jennings,
837 S., Maury, O., Lotze, H.K., 2019. Twenty-first-century climate change impacts on marine animal biomass
838 and ecosystem structure across ocean basins. *Glob. Chang. Biol.* 25, 459–472.
839 <https://doi.org/10.1111/gcb.14512>

840 Budge, S., Springer, A., SJ, I., Sheffield, G., 2007. Fatty acid biomarkers reveal niche separation in an Arctic
841 benthic food web. *Mar. Ecol. Prog. Ser.* 336, 305–309. <https://doi.org/10.3354/meps336305>

842 Budge, S.M., Iverson, S.J., Bowen, W.D., Ackman, R.G., 2002. Among- and within-species variability in fatty
843 acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of
844 St. Lawrence. *Can. J. Fish. Aquat. Sci.* 59, 886–898. <https://doi.org/10.1139/f02-062>

845 Budge, S.M., Parrish, C.C., 1998. Lipid biogeochemistry of plankton, settling matter and sediments in Trinity
846 Bay, Newfoundland. I. Lipid classes. *Org. Geochem.* 29, 1531–1545. <https://doi.org/10.1016/S0146->
847 6380(98)00176-4

848 Campus, M., Addis, M.F., Cappuccinelli, R., Porcu, M.C., Pretti, L., Tedde, V., Secchi, N., Stara, G., Roggio, T.,
849 2010. Stress relaxation behaviour and structural changes of muscle tissues from Gilthead Sea Bream
850 (*Sparus aurata*) following high pressure treatment. *J. Food Eng.* 96, 192–198.
851 <https://doi.org/10.1016/j.jfoodeng.2009.07.013>

852 Carbajal, A., Monclús, L., Tallo-Parra, O., Sabes-Alsina, M., Vinyoles, D., Lopez-Bejar, M., 2018. Cortisol
853 detection in fish scales by enzyme immunoassay: Biochemical and methodological validation. *J. Appl.*
854 *Ichthyol.* 34, 967–970. <https://doi.org/10.1111/jai.13674>

855 Celandier, M.C., 2011. Cocktail effects on biomarker responses in fish. *Aquat. Toxicol.* 105, 72–77.
856 <https://doi.org/10.1016/j.aquatox.2011.06.002>

857 Cerezuela, R., Guardiola, F.A., Cuesta, A., Esteban, M.Á., 2016. Enrichment of gilthead seabream (*Sparus*
858 *aurata*) diet with palm fruit extracts and probiotics: Effects on skin mucosal immunity. *Fish Shellfish*
859 *Immunol.* 49, 100–109. <https://doi.org/10.1016/j.fsi.2015.12.028>

860 Chassot, E., Bonhommeau, S., Dulvy, N.K., Mélin, F., Watson, R., Gascuel, D., Le Pape, O., 2010. Global
861 marine primary production constrains fisheries catches. *Ecol. Lett.* 13, 495–505.
862 <https://doi.org/10.1111/j.1461-0248.2010.01443.x>

863 Chouvelon, T., Brach-Papa, C., Auger, D., Bodin, N., Bruzac, S., Crochet, S., Degroote, M., Hollanda, S.J.,
864 Hubert, C., Knoery, J., Munsch, C., Puech, A., Rozuel, E., Thomas, B., West, W., Bourjea, J., Nikolic,
865 N., 2017. Chemical contaminants (trace metals, persistent organic pollutants) in albacore tuna from
866 western Indian and south-eastern Atlantic Oceans: Trophic influence and potential as tracers of
867 populations. *Sci. Total Environ.* 596–597, 481–495. <https://doi.org/10.1016/j.scitotenv.2017.04.048>

868 Chouvelon, T., Caurant, F., Cherel, Y., Simon-Bouhet, B., Spitz, J., Bustamante, P., 2014. Species- and size-
869 related patterns in stable isotopes and mercury concentrations in fish help refine marine ecosystem
870 indicators and provide evidence for distinct management units for hake in the Northeast Atlantic. *ICES J.*
871 *Mar. Sci.* 71, 1073–1087. <https://doi.org/10.1093/icesjms/fst199>

872 Cichon, M., 1997. Evolution of longevity through optimal resource allocation. *Proc. R. Soc. B Biol. Sci.* 264,
873 1383–1388. <https://doi.org/10.1098/rspb.1997.0192>

874 Cohen, S., Janicki-Deverts, D., Doyle, W.J., Miller, G.E., Frank, E., Rabin, B.S., Turner, R.B., 2012. Chronic
875 stress, glucocorticoid receptor resistance, inflammation, and disease risk. *Proc. Natl. Acad. Sci.* 109,
876 5995–5999. <https://doi.org/10.1073/pnas.1118355109>

877 Costantini, D., Marasco, V., Møller, A.P., 2011. A meta-analysis of glucocorticoids as modulators of oxidative
878 stress in vertebrates. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.*
879 <https://doi.org/10.1007/s00360-011-0566-2>

880 Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in
881 conservation. *Proc. R. Soc. B Biol. Sci.* 283, 20152592. <https://doi.org/10.1098/rspb.2015.2592>

882 Crain, C.M., Kroeker, K., Halpern, B.S., 2008. Interactive and cumulative effects of multiple human stressors in
883 marine systems. *Ecol. Lett.* 11, 1304–1315. <https://doi.org/10.1111/j.1461-0248.2008.01253.x>

884 Cresson, P., Bouchoucha, M., Miralles, F., Elleboode, R., Mahé, K., Maruszczak, N., Thebault, H., Cossa, D.,
885 2015. Are red mullet efficient as bio-indicators of mercury contamination? A case study from the French
886 Mediterranean. *Mar. Pollut. Bull.* 91, 191–199. <https://doi.org/10.1016/j.marpolbul.2014.12.005>

887 Cresson, P., Fabri, M.C., Miralles, F.M., Dufour, J.L., Elleboode, R., Sevin, K., Mahé, K., Bouchoucha, M.,
888 2016. Variability of PCB burden in 5 fish and sharks species of the French Mediterranean continental
889 slope. *Environ. Pollut.* 212, 374–381. <https://doi.org/10.1016/j.envpol.2016.01.044>

890 Cuesta, A., Laiz-Carrión, R., Martín del Río, M.P., Meseguer, J., Miguel Mancera, J., Ángeles Esteban, M.,
891 2005. Salinity influences the humoral immune parameters of gilthead seabream (*Sparus aurata*). *Fish*
892 *Shellfish Immunol.* 18, 255–261. <https://doi.org/10.1016/j.fsi.2004.07.009>

893 Cushing, D.H., Horwood, J.W., 1994. The growth and death of fish larvae. *J. Plankton Res.* 16, 291–300.
894 <https://doi.org/10.1093/plankt/16.3.291>

895 Dalsgaard, J., St. John, M., Kattner, G., Muller-Navarra, D., Hagen, W., 2003. Fatty acids as trophic markers in
896 the pelagic marine environment, *Advances In Marine Biology*.

897 Davis, A.K., Maney, D.L., Maerz, J.C., 2008. The use of leukocyte profiles to measure stress in vertebrates: a
898 review for ecologists. *Funct. Ecol.* 22, 760–772. <https://doi.org/10.1111/j.1365-2435.2008.01467.x>

899 Denver, R.J., 2009. Structural and functional evolution of vertebrate neuroendocrine stress systems. *Ann. N. Y.*
900 *Acad. Sci.* 1163, 1–16. <https://doi.org/10.1111/j.1749-6632.2009.04433.x>

901 Derolez, V., Malet, N., Fiandrino, A., Lagarde, F., Richard, M., Ouisse, V., Bec, B., Aliaume, C., 2020. Fifty
902 years of ecological changes: Regime shifts and drivers in a coastal Mediterranean lagoon during
903 oligotrophication. *Sci. Total Environ.* 732, 139292. <https://doi.org/10.1016/j.scitotenv.2020.139292>

904 Development Core Team, R., 2008. R: A Language and Environment for Statistical Computing.

- 905 Di Giulio, R., Meyer, J., 2008. Reactive oxygen species and oxidative stress, in: Di Giulio, R., Hinton, D. (Eds.),
906 The Toxicology of Fishes. CRC Press, Taylor and Francis.
- 907 Díaz-Rosales, P., Salinas, I., Rodríguez, A., Cuesta, A., Chabrilón, M., Balebona, M.C., Morinigo, M.A.,
908 Esteban, M.A., Meseguer, J., 2006. Gilthead seabream (*Sparus aurata* L.) innate immune response after
909 dietary administration of heat-inactivated potential probiotics. *Fish Shellfish Immunol.* 20, 482–492.
910 <https://doi.org/10.1016/j.fsi.2005.06.007>
- 911 Doney, S.C., Ruckelshaus, M., Emmett Duffy, J., Barry, J.P., Chan, F., English, C.A., Galindo, H.M.,
912 Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley,
913 L.D., 2012. Climate Change Impacts on Marine Ecosystems. *Ann. Rev. Mar. Sci.* 4, 11–37.
914 <https://doi.org/10.1146/annurev-marine-041911-111611>
- 915 El-Sabaawi, R., Dower, J.F., Kainz, M., Mazumder, A., 2009. Interannual variability in fatty acid composition of
916 the copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia. *Mar. Ecol. Prog. Ser.* 382,
917 151–161. <https://doi.org/10.3354/meps07915>
- 918 Ellis, A.E., 1990. Lysozyme Assays, in: Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S. and Van
919 Muiswinkel, W.B. (Ed.), *Techniques in Fish Immunology*. pp. 101–103.
- 920 Ericsson, C., Nistér, M., 2011. Protein Extraction from Solid Tissue, in: Joakim Dillner (Ed.), *Methods in*
921 *Biobanking*. Humana Press, Totowa, NJ, Totowa, NJ, pp. 307–312. [https://doi.org/10.1007/978-1-59745-](https://doi.org/10.1007/978-1-59745-423-0_17)
922 [423-0_17](https://doi.org/10.1007/978-1-59745-423-0_17)
- 923 Escalas, A., Ferraton, F., Paillon, C., Vidy, G., Carcaillet, F., Salen-Picard, C., Le Loc'h, F., Richard, P.,
924 Darnaude, A.M., 2015. Spatial variations in dietary organic matter sources modulate the size and
925 condition of fish juveniles in temperate lagoon nursery sites. *Estuar. Coast. Shelf Sci.* 152, 78–90.
926 <https://doi.org/10.1016/j.ecss.2014.11.021>
- 927 Folch, J., Lees, M., Sloane Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids
928 from animal tissues. *J. Biol. Chem.* 226, 497–509.
- 929 Gaetke, L.M., Chow, C.K., 2003. Copper toxicity, oxidative stress, and antioxidant nutrients. *Toxicology* 189,
930 147–163. [https://doi.org/10.1016/S0300-483X\(03\)00159-8](https://doi.org/10.1016/S0300-483X(03)00159-8)
- 931 Gao, Q., Shin, P., Lin, G., Chen, S., Cheung, S., 2006. Stable isotope and fatty acid evidence for uptake of
932 organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. *Mar. Ecol. Prog.*
933 *Ser.* 317, 273–283. <https://doi.org/10.3354/meps317273>
- 934 García-Navarro, J.A., Franco, L., Romero, D., 2017. Differences in the accumulation and tissue distribution of
935 Pb, Cd, and Cu in Mediterranean mussels (*Mytilus galloprovincialis*) exposed to single, binary, and
936 ternary metal mixtures. *Environ. Sci. Pollut. Res.* 24, 6599–6610. [https://doi.org/10.1007/s11356-016-](https://doi.org/10.1007/s11356-016-8349-7)
937 [8349-7](https://doi.org/10.1007/s11356-016-8349-7)
- 938 Goikoetxea, A., Sadoul, B., Blondeau-Bidet, E., Aerts, J., Blanc, M.O., Parrinello, H., Barrachina, C., Pratlong,
939 M., Geffroy, B., 2021. Genetic pathways underpinning hormonal stress responses in fish exposed to
940 short- and long-term warm ocean temperatures. *Ecol. Indic.* 120, 106937.
941 <https://doi.org/10.1016/j.ecolind.2020.106937>
- 942 Goldan, O., Popper, D., Karplus, I., 2008. Food competition in small groups of juvenile gilthead sea bream
943 (*Sparus aurata*). *Isr J Aquacult* 60, 128–133. <https://doi.org/http://hdl.handle.net/10524/19075>
- 944 Graeve, M., Kattner, G., Piepenburg, D., 1997. Lipids in arctic benthos: Does the fatty acid and alcohol
945 composition reflect feeding and trophic interactions? *Polar Biol.* 18, 53–61.
946 <https://doi.org/10.1007/s003000050158>
- 947 Guardiola, F.A., Chaves-Pozo, E., Espinosa, C., Romero, D., Meseguer, J., Cuesta, A., Esteban, M.A., 2016.
948 Mercury accumulation, structural damages, and antioxidant and immune status changes in the gilthead
949 seabream (*sparus aurata* L.) exposed to methylmercury. *Arch. Environ. Contam. Toxicol.* 70, 734–746.
950 <https://doi.org/10.1007/s00244-016-0268-6>
- 951 Guardiola, F.A., Dioguardi, M., Parisi, M.G., Trapani, M.R., Meseguer, J., Cuesta, A., Cammarata, M., Esteban,
952 M.A., 2015. Evaluation of waterborne exposure to heavy metals in innate immune defences present on
953 skin mucus of gilthead seabream (*Sparus aurata*). *Fish Shellfish Immunol.* 45, 112–123.
954 <https://doi.org/10.1016/j.fsi.2015.02.010>
- 955 Harmelin-Vivien, M., Bodiguel, X., Charmasson, S., Loizeau, V., Mellon-Duval, C., Tronczyński, J., Cossa, D.,
956 2012. Differential biomagnification of PCB, PBDE, Hg and Radiocesium in the food web of the
957 European hake from the NW Mediterranean. *Mar. Pollut. Bull.* 64, 974–983.
958 <https://doi.org/10.1016/j.marpolbul.2012.02.014>
- 959 Harris, B.N., 2020. Stress hypothesis overload: 131 hypotheses exploring the role of stress in tradeoffs,
960 transitions, and health. *Gen. Comp. Endocrinol.* 288, 113355.
961 <https://doi.org/10.1016/j.ygcen.2019.113355>
- 962 Heather, F.J., Childs, D.Z., Darnaude, A.M., Blanchard, J.L., 2018. Using an integral projection model to assess
963 the effect of temperature on the growth of gilthead seabream *Sparus aurata*. *PLoS One* 13, 1–19.
964 <https://doi.org/10.1371/journal.pone.0196092>

- 965 Ifremer, 2009. Panorama général. Réseau de Suivi Lagunaire du Languedoc-Roussillon. Sète, France.
- 966 Inness, C.L.W., Metcalfe, N.B., 2008. The impact of dietary restriction, intermittent feeding and compensatory
967 growth on reproductive investment and lifespan in a short-lived fish. *Proc. R. Soc. B Biol. Sci.* 275,
968 1703–1708. <https://doi.org/10.1098/rspb.2008.0357>
- 969 Isnard, E., Tournois, J., McKenzie, D.J., Ferraton, F., Bodin, N., Aliaume, C., Darnaude, A.M., 2015. Getting a
970 good start in life? A comparative analysis of the quality of lagoons as juvenile habitats for the gilthead
971 seabream *Sparus aurata* in the Gulf of Lions. *Estuaries and Coasts* 38, 1937–1950.
972 <https://doi.org/10.1007/s12237-014-9939-6>
- 973 Izquierdo, M., 2005. Essential fatty acid requirements in Mediterranean fish species 102, 91–102.
- 974 Johnson, E.O., Kamilaris, T.C., Chrousos, G.P., Gold, P.W., 1992. Mechanisms of stress: A dynamic overview
975 of hormonal and behavioral homeostasis. *Neurosci. Biobehav. Rev.* 16, 115–130.
976 [https://doi.org/10.1016/S0149-7634\(05\)80175-7](https://doi.org/10.1016/S0149-7634(05)80175-7)
- 977 Joseph, J.D., 1982. Lipid composition of marine and estuarine invertebrates. Part II: Mollusca. *Prog. Lipid Res.*
978 21, 109–153. [https://doi.org/10.1016/0163-7827\(82\)90002-9](https://doi.org/10.1016/0163-7827(82)90002-9)
- 979 Kadiiska, M.B., Mason, R.P., 2002. In vivo copper-mediated free radical production: an ESR spin-trapping
980 study. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* 58, 1227–1239. [https://doi.org/10.1016/S1386-1425\(01\)00713-2](https://doi.org/10.1016/S1386-1425(01)00713-2)
- 982 Kattner, G., Graeve, M., Hagen, W., 2012. Energy reserves of Southern Ocean copepods: Triacylglycerols with
983 unusually long-chain monounsaturated fatty acids. *Mar. Chem.* 138–139, 7–12.
984 <https://doi.org/10.1016/j.marchem.2012.05.002>
- 985 Kelly, J.R., Scheibling, R.E., 2012. Fatty acids as dietary tracers in benthic food webs. *Mar. Ecol. Prog. Ser.* 446,
986 1–22. <https://doi.org/10.3354/meps09559>
- 987 Koussoroplis, A.-M., Bec, A., Perga, M.-E., Koutrakis, E., Bourdier, G., Desvillettes, C., 2011. Fatty acid transfer
988 in the food web of a coastal Mediterranean lagoon: Evidence for high arachidonic acid retention in fish.
989 *Estuar. Coast. Shelf Sci.* 91, 450–461. <https://doi.org/10.1016/j.ecss.2010.11.010>
- 990 Koven, W., Van Anholt, R., Lutzky, S., Atia, I. Ben, Nixon, O., Ron, B., Tandler, A., 2003. The effect of dietary
991 arachidonic acid on growth, survival, and cortisol levels in different-age gilthead seabream larvae (*Sparus*
992 *aurata*) exposed to handling or daily salinity change. *Aquaculture* 228, 307–320.
993 [https://doi.org/10.1016/S0044-8486\(03\)00317-X](https://doi.org/10.1016/S0044-8486(03)00317-X)
- 994 Laiz-Carrión, R., Sangiao-Alvarellos, S., Guzmán, J.M., Martín Del Río, M.P., Soengas, J.L., Mancera, J.M.,
995 2005. Growth performance of gilthead sea bream *Sparus aurata* in different osmotic conditions:
996 Implications for osmoregulation and energy metabolism. *Aquaculture* 250, 849–861.
997 <https://doi.org/10.1016/j.aquaculture.2005.05.021>
- 998 Le Fur, I., De Wit, R., Plus, M., Oheix, J., Derolez, V., Simier, M., Malet, N., Ouisse, V., 2019. Re-
999 oligotrophication trajectories of macrophyte assemblages in Mediterranean coastal lagoons based on 17-
1000 year time-series. *Mar. Ecol. Prog. Ser.* 608, 13–32. <https://doi.org/10.3354/meps12814>
- 1001 Lee, B.G., Wallace, W.G., Luoma, S.N., 1998. Uptake and loss kinetics of Cd, Cr and Zn in the bivalves
1002 *Potamocorbula amurensis* and *Macoma balthica*: Effects of size and salinity. *Mar. Ecol. Prog. Ser.* 175,
1003 177–189. <https://doi.org/10.3354/meps175177>
- 1004 Lee, W.-S., Monaghan, P., Metcalfe, N.B., 2012. The pattern of early growth trajectories affects adult breeding
1005 performance. *Ecology* 93, 902–912. <https://doi.org/10.1890/11-0890.1>
- 1006 Lemonnier, C., Bize, P., Boonstra, R., Dobson, F.S., Criscuolo, F., Viblanc, V.A., 2022. Effects of the social
1007 environment on vertebrate fitness and health in nature: Moving beyond the stress axis. *Horm. Behav.* 145,
1008 105232. <https://doi.org/10.1016/j.yhbeh.2022.105232>
- 1009 Liaquat, L., Sadir, S., Batool, Z., Tabassum, S., Shahzad, S., Afzal, A., Haider, S., 2019. Acute aluminum
1010 chloride toxicity revisited: Study on DNA damage and histopathological, biochemical and neurochemical
1011 alterations in rat brain. *Life Sci.* 217, 202–211. <https://doi.org/S0024320518307926>
- 1012 Losso, C., Ghirardini, A.V., 2010. Overview of ecotoxicological studies performed in the Venice Lagoon (Italy).
1013 *Environ. Int.* 36, 92–121. <https://doi.org/10.1016/j.envint.2009.07.017>
- 1014 Lotze, H.K., 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* (80-.).
1015 312, 1806–1809. <https://doi.org/10.1126/science.1128035>
- 1016 Lovern, J.A., 1935. Fat metabolism in fishes: The fats of some plankton crustacea. *Biochem. J.* 29, 847–9.
- 1017 Maazouzi, C., Masson, G., Izquierdo, M.S., Pihan, J.C., 2007. Fatty acid composition of the amphipod
1018 *Dikerogammarus villosus*: Feeding strategies and trophic links. *Comp. Biochem. Physiol. - A Mol. Integr.*
1019 *Physiol.* 147, 868–875. <https://doi.org/10.1016/j.cbpa.2007.02.010>
- 1020 MacDougall-Shackleton, S.A., Bonier, F., Romero, L.M., Moore, I.T., 2019. Glucocorticoids and “Stress” Are
1021 Not Synonymous. *Integr. Org. Biol.* 1. <https://doi.org/10.1093/iob/obz017>
- 1022 Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., Diniz, M.S., 2013. Influence of temperature in thermal and
1023 oxidative stress responses in estuarine fish. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 166,
1024 237–243. <https://doi.org/10.1016/j.cbpa.2013.06.008>

- 1025 Madeira, D., Vinagre, C., Diniz, M.S., 2016. Are fish in hot water? Effects of warming on oxidative stress
1026 metabolism in the commercial species *Sparus aurata*. *Ecol. Indic.* 63, 324–331.
1027 <https://doi.org/10.1016/j.ecolind.2015.12.008>
- 1028 Mahé, K., Bellail, R., Dufour, J.L., Boiron-Ieroy, A., Duhamel, E., Elleboode, R., Félix, J., Grellier, P., Huet, J.,
1029 Labastie, J., Roy, D. Le, Lizaud, O., Line, M., Martin, S., Metral, L., Nédelec, D., Vérin, Y., Badts, V.,
1030 2009. French summary of age estimation procedures.
- 1031 Martin, L.B., 2009. Stress and immunity in wild vertebrates: Timing is everything. *Gen. Comp. Endocrinol.* 163,
1032 70–76. <https://doi.org/10.1016/j.ygcen.2009.03.008>
- 1033 McEwen, B. S., & Wingfield, J. C. 2003. The concept of allostasis in biology and biomedicine. *Hormones and*
1034 *behavior*, 43(1), 2-15.
- 1035 McKenzie, D.J., Axelsson, M., Chabot, D., Claireaux, G., Cooke, S.J., Corner, R.A., De Boeck, G., Domenici,
1036 P., Guerreiro, P.M., Hamer, B., Jørgensen, C., Killen, S.S., Lefevre, S., Marras, S., Michaelidis, B.,
1037 Nilsson, G.E., Peck, M.A., Perez-Ruzafa, A., Rijnsdorp, A.D., Shiels, H.A., Steffensen, J.F., Svendsen,
1038 J.C., Svendsen, M.B.S., Teal, L.R., van der Meer, J., Wang, T., Wilson, J.M., Wilson, R.W., Metcalfe,
1039 J.D., 2016. Conservation physiology of marine fishes: state of the art and prospects for policy. *Conserv.*
1040 *Physiol.* 4, cow046. <https://doi.org/10.1093/conphys/cow046>
- 1041 Médale, F., Kaushik, S., Sitjà-Bobadilla, A., Peña-Llopis, S., Gómez-Requeni, P., Médale, F., Kaushik, S.,
1042 Pérez-Sánchez, J., 2005. Effect of fish meal replacement by plant protein sources on non-specific defence
1043 mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). *Aquaculture* 249, 387–400.
1044 <https://doi.org/10.1016/j.aquaculture.2005.03.031>
- 1045 Mercier, L., Mouillot, D., Bruguier, O., Vigliola, L., Darnaude, A.M., 2012. Multi-element otolith fingerprints
1046 unravel sea-lagoon lifetime migrations of gilthead sea bream *Sparus aurata*. *Mar. Ecol. Prog. Ser.* 444,
1047 175–194. <https://doi.org/10.3354/meps09444>
- 1048 Merry, B., 1995. Effect of dietary restriction on aging – an update. *Rev. Clin. Gerontol.* 5, 247.
1049 <https://doi.org/10.1017/S0959259800004305>
- 1050 Metcalfe, N.B., Alonso-Alvarez, C., 2010. Oxidative stress as a life-history constraint: The role of reactive
1051 oxygen species in shaping phenotypes from conception to death. *Funct. Ecol.* 24, 984–996.
1052 <https://doi.org/10.1111/j.1365-2435.2010.01750.x>
- 1053 Metcalfe, N.B., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* 16,
1054 254–260. [https://doi.org/10.1016/S0169-5347\(01\)02124-3](https://doi.org/10.1016/S0169-5347(01)02124-3)
- 1055 Mille, T., Cresson, P., Chouvelon, T., Bustamante, P., Brach-Papa, C., Sandrine, B., Rozuel, E., Bouchouha,
1056 M., 2018. Trace metal concentrations in the muscle of seven marine species: Comparison between the
1057 Gulf of Lions (North-West Mediterranean Sea) and the Bay of Biscay (North-East Atlantic Ocean). *Mar.*
1058 *Pollut. Bull.* 135, 9–16. <https://doi.org/10.1016/j.marpolbul.2018.05.051>
- 1059 Monaghan, P., Metcalfe, N.B., Torres, R., 2009. Oxidative stress as a mediator of life history trade-offs:
1060 Mechanisms, measurements and interpretation. *Ecol. Lett.* 12, 75–92. <https://doi.org/10.1111/j.1461-0248.2008.01258.x>
- 1061
- 1062 Morgan, I.J., McCarthy, I.D., Metcalfe, N.B., 2000. Life-history strategies and protein metabolism in
1063 overwintering juvenile Atlantic salmon: Growth is enhanced in early migrants through lower protein
1064 turnover. *J. Fish Biol.* 56, 637–647. <https://doi.org/10.1006/jfbi.1999.1183>
- 1065 Mourente, G., Díaz-Salvago, E., Tocher, D.R., Bell, J.G., 2000. Effects of dietary polyunsaturated fatty
1066 acid/vitamin E (PUFA/tocopherol ratio on antioxidant defence mechanisms of juvenile gilthead sea bream
1067 (*Sparus aurata* L., *Osteichthyes*, *Sparidae*). *Fish Physiol. Biochem.* 23, 337–351.
1068 <https://doi.org/10.1023/A:1011128510062>
- 1069 Mourente, G., Diaz-Salvago, E., Bell, J.G., Tocher, D.R., 2002. Increased activities of hepatic antioxidant
1070 defence enzymes in juvenile gilthead sea bream (*Sparus aurata* L.) fed dietary oxidised oil: Attenuation
1071 by dietary vitamin E. *Aquaculture* 214, 343–361. [https://doi.org/10.1016/S0044-8486\(02\)00064-9](https://doi.org/10.1016/S0044-8486(02)00064-9)
- 1072 Munaron, D., Mérigot, B., Derolez, V., Tapie, N., Budzinski, H., Fiandrino, A., 2023. Evaluating pesticide
1073 mixture risks in French Mediterranean coastal lagoons waters. *Sci. Total Environ.* 867, 161303.
1074 <https://doi.org/10.1016/j.scitotenv.2022.161303>
- 1075 Munck, A., Guyre, P.M., Holbrook, N.J., 1984. Physiological functions of glucocorticoids in stress and their
1076 relation to pharmacological actions. *Endocr. Rev.* 5, 25–44. <https://doi.org/10.1210/edrv-5-1-25>
- 1077 Naccari, C., Cicero, N., Ferrantelli, V., Giangrosso, G., Vella, A., Macaluso, A., Naccari, F., Dugo, G., 2015.
1078 Toxic metals in pelagic, benthic and demersal fish species from Mediterranean FAO Zone 37. *Bull.*
1079 *Environ. Contam. Toxicol.* 95, 567–573. <https://doi.org/10.1007/s00128-015-1585-6>
- 1080 Navaratnarajah, A., Jackson, S.H.D., 2013. The physiology of ageing. *Medicine (Baltimore)*. 41, 5–8.
1081 <https://doi.org/10.1016/j.mpmed.2012.10.009>
- 1082 Nixon, S.W., Buckley, B.A., Granger, S.L., Entsua-Mensah, M., Ansa-Asare, O., White, M.J., McKinney, R.A.,
1083 Mensah, E., 2007. Anthropogenic enrichment and nutrients in some tropical lagoons of Ghana, west
1084 Africa. *Ecol. Appl.* 17, S144–S164. <https://doi.org/10.1890/05-0684.1>

1085 Noh, K.-M., Koh, J.-Y., 2000. Induction and Activation by Zinc of NADPH Oxidase in Cultured Cortical
1086 Neurons and Astrocytes. *J. Neurosci.* 20, RC111. <https://doi.org/10.1523/JNEUROSCI.20-23-j0001.2000>
1087 Ourgaud, M., Ruitton, S., Bourgoigne, H., Bustamante, P., Churlaud, C., Guillou, G., Lebreton, B., Harmelin-
1088 Vivien, M.L., 2018. Trace elements in a Mediterranean scorpaenid fish: Bioaccumulation processes and
1089 spatial variations. *Prog. Oceanogr.* 163, 184–195. <https://doi.org/10.1016/j.pocean.2017.11.008>
1090 Parrish, C.C., 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples, in: *Lipids in*
1091 *Freshwater Ecosystems*. Springer New York, pp. 4–20. https://doi.org/10.1007/978-1-4612-0547-0_2
1092 Pérez-Ruzafa, A., Marcos, C., Pérez-Ruzafa, I.M., Pérez-Marcos, M., 2011. Coastal lagoons: “transitional
1093 ecosystems” between transitional and coastal waters. *J. Coast. Conserv.* 15, 369–392.
1094 <https://doi.org/10.1007/s11852-010-0095-2>
1095 Pernet, F., 2016. Lipides marins et fonctionnement des écosystèmes et des organismes. Univ. Bretagne Occident.
1096 Pommier, J., Frenette, J.J., Glémet, H., 2010. Relating RNA:DNA ratio in *Eurytemora affinis* to seston fatty
1097 acids in a highly dynamic environment. *Mar. Ecol. Prog. Ser.* 400, 143–154.
1098 <https://doi.org/10.3354/meps08413>
1099 Portner, H.O., Farrell, A.P., 2008. ECOLOGY: Physiology and Climate Change. *Science* (80-.). 322, 690–692.
1100 <https://doi.org/10.1126/science.1163156>
1101 Prato, E., Danieli, A., Maffia, M., Biandolino, F., 2012. Lipid Contents and Fatty Acid Compositions of *Idotea*
1102 *baltica* and *Sphaeroma serratum* (Crustacea: Isopoda) as Indicators of Food Sources. *Zool. Stud.* 51, 38–
1103 50.
1104 Purves, D., Scharlemann, J.P., Harfoot, M., Newbold, T., Tittensor, D.P., Hutton, J., Emmott, S., 2013.
1105 Ecosystems: time to model all life on Earth. *Nature* 493, 295. <https://doi.org/10.1038/493295a>
1106 Queiros, Q., Fromentin, J.M., Gasset, E., Dutto, G., Huiban, C., Metral, L., Leclerc, L., Schull, Q., McKenzie,
1107 D.J., Saraux, C., 2019. Food in the sea: Size also matters for pelagic fish. *Front. Mar. Sci.* 6, 385.
1108 <https://doi.org/10.3389/fmars.2019.00385>
1109 Richards, J.G., Curtis, P.J., Burnison, B.K., Playle, R.C., 2001. Effects of natural organic matter source on
1110 reducing metal toxicity to rainbow trout (*Oncorhynchus mykiss*) and on metal binding to their gills.
1111 *Environ. Toxicol. Chem.* 20, 1159–1166. <https://doi.org/10.1002/etc.5620200604>
1112 Rink, L., Kirchner, H., 2000. Zinc-altered immune function and cytokine production. *J. Nutr.* 130, 1407S-1411S.
1113 <https://doi.org/10.1093/jn/130.5.1407S>
1114 Rollo, C.D., 2002. Growth negatively impacts the life span of mammals. *Evol. Dev.* 4, 55–61.
1115 Romero, L.M., 2004. Physiological stress in ecology: Lessons from biomedical research. *Trends Ecol. Evol.* 19,
1116 249–255. <https://doi.org/10.1016/j.tree.2004.03.008>
1117 Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The Reactive Scope Model - a new model integrating
1118 homeostasis, allostasis, and stress. *Horm. Behav.* 55, 375–89.
1119 <https://doi.org/10.1016/j.yhbeh.2008.12.009>
1120 Rosa, R., Seibel, B.A., 2008. Synergistic effects of climate-related variables suggest future physiological
1121 impairment in a top oceanic predator. *Proc. Natl. Acad. Sci.* 105, 20776–20780.
1122 <https://doi.org/10.1073/pnas.0806886105>
1123 Rosecchi, E., 1987. L'alimentation de *Diplodus annularis*, *Diplodus sargus*, *Diplodus vulgaris* et *Sparus aurata*
1124 (*pisces, sparidae*) dans le Golfe du Lion et les lagunes littorales. *Reu. Trau. Inst. Pêches marit.* 49, 125–
1125 141.
1126 Sadoul, B., Geffroy, B., 2019. Measuring cortisol, the major stress hormone in fishes. *J. Fish Biol.* 94, 540–555.
1127 <https://doi.org/10.1111/jfb.13904>
1128 Saera-Vila, A., Benedito-Palos, L., Sitjà-Bobadilla, A., Nàcher-Mestre, J., Serrano, R., Kaushik, S., Pérez-
1129 Sánchez, J., 2009. Assessment of the health and antioxidant trade-off in gilthead sea bream (*Sparus*
1130 *aurata* L.) fed alternative diets with low levels of contaminants. *Aquaculture* 296, 87–95.
1131 <https://doi.org/10.1016/j.aquaculture.2009.07.028>
1132 Salati, F., Roncarati, A., Angelucci, G., Fenza, A., Meluzzi, A., 2016. Stress and Humoral Innate Immune
1133 Response of Gilthead Seabream *Sparus aurata* Cultured in Sea Cages. *J. Aquat. Anim. Health* 28, 166–
1134 172. <https://doi.org/10.1080/08997659.2016.1173604>
1135 Sanchez, G., 2013. PLS Path Modeling with R, Trowchez E. ed. <https://doi.org/citeulike-article-id:13341888>
1136 Saraux, C., Van Beveren, E., Brosset, P., Queiros, Q., Bourdeix, J.H., Dutto, G., Gasset, E., Jac, C.,
1137 Bonhommeau, S., Fromentin, J.M., 2019. Small pelagic fish dynamics: A review of mechanisms in the
1138 Gulf of Lions. *Deep. Res. Part II Top. Stud. Oceanogr.* 159, 52–61.
1139 <https://doi.org/10.1016/j.dsr2.2018.02.010>
1140 Sardenne, F., Bodin, N., Metral, L., Crottier, A., Le Grand, F., Bideau, A., Brisset, B., Bourjea, J., Saraux, C.,
1141 Bonhommeau, S., Kerzérho, V., Bernard, S., Rouyer, T., 2019. Effects of extraction method and storage
1142 of dry tissue on marine lipids and fatty acids. *Anal. Chim. Acta* 1051, 82–93.
1143 <https://doi.org/10.1016/j.aca.2018.11.012>

- 1144 Scharf, F.S., Juanes, F., Rountree, R.A., 2000. Predator size - Prey size relationships of marine fish predators:
1145 Interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Mar. Ecol. Prog.*
1146 *Ser.* 208, 229–248. <https://doi.org/10.3354/meps208229>
- 1147 Schmitz, G., Ecker, J., 2008. The opposing effects of n-3 and n-6 fatty acids. *Prog. Lipid Res.* 47, 147–155.
1148 <https://doi.org/10.1016/j.plipres.2007.12.004>
- 1149 Schwartz, M.L., Curtis, P.J., Playle, R.C., 2004. Influence of natural organic matter source on acute copper, lead,
1150 and cadmium toxicity to rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* 23, 2889–2899.
1151 <https://doi.org/10.1897/03-561.1>
- 1152 Sevcikova, M., Modra, H., Slaninova, A., Svobodova, Z., 2011. Metals as a cause of oxidative stress in fish: a
1153 review. *Vet. Med. (Praha)*. 56, 537–546. <https://doi.org/10.17221/4272-VETMED>
- 1154 Sitjà-Bobadilla, A., Mingarro, M., Pujalte, M.J., Garay, E., Alvarez-Pellitero, P., Pérez-Sánchez, J., 2003.
1155 Immunological and pathological status of gilthead sea bream (*Sparus aurata* L.) under different long-term
1156 feeding regimes. *Aquaculture* 220, 707–724. [https://doi.org/10.1016/S0044-8486\(02\)00615-4](https://doi.org/10.1016/S0044-8486(02)00615-4)
- 1157 Soares, S.S., Martins, H., Gutiérrez-Merino, C., Aureliano, M., 2008. Vanadium and cadmium in vivo effects in
1158 teleost cardiac muscle: Metal accumulation and oxidative stress markers. *Comp. Biochem. Physiol. - C*
1159 *Toxicol. Pharmacol.* 147, 168–178. <https://doi.org/10.1016/j.cbpc.2007.09.003>
- 1160 Sogard, S.M., 1997. Size-selective mortality in the juvenile stage of teleost fishes : a review. *Bull. Mar. Sci.* 60,
1161 1129–1157.
- 1162 St John, M., Lund, T., 1996. Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced
1163 condition of juvenile North Sea cod. *Mar. Ecol. Prog. Ser.* 131, 75–85.
1164 <https://doi.org/10.3354/meps131075>
- 1165 Stearns, S., 1989. Trade-offs in life-history evolution. *Funct. Ecol.* 3, 259–268. <https://doi.org/10.2307/2389364>
- 1166 Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* 18,
1167 321–336. [https://doi.org/10.1016/0891-5849\(94\)00159-H](https://doi.org/10.1016/0891-5849(94)00159-H)
- 1168 Tandler, A., Anav, F.A., Choshniak, I., 1995. The effect of salinity on growth rate, survival and swimbladder
1169 inflation in gilthead seabream, *Sparus aurata*, larvae. *Aquaculture* 135, 343–353.
1170 [https://doi.org/10.1016/0044-8486\(95\)01029-7](https://doi.org/10.1016/0044-8486(95)01029-7)
- 1171 Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish metabolism and functions
1172 of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* 11, 107–184. <https://doi.org/10.1080/713610925>
- 1173 Todgham, A.E., Stillman, J.H., 2013. Physiological responses to shifts in multiple environmental stressors:
1174 Relevance in a changing world. *Integr. Comp. Biol.* 53, 539–544. <https://doi.org/10.1093/icb/ict086>
- 1175 Tomasello, B., Copat, C., Pulvirenti, V., Ferrito, V., Ferrante, M., Renis, M., Sciacca, S., Tigano, C., 2012.
1176 Biochemical and bioaccumulation approaches for investigating marine pollution using Mediterranean
1177 rainbow wrasse, *Coris julis* (Linnaeus 1798). *Ecotoxicol. Environ. Saf.* 86, 168–175.
1178 <https://doi.org/10.1016/j.ecoenv.2012.09.012>
- 1179 Tort, L., Padros, F., Rotllant, J., Crespo, S., 1998. Winter syndrome in the gilthead sea bream *Sparus aurata*.
1180 Immunological and histopathological features. *Fish Shellfish Immunol.* 8, 37–47.
- 1181 Tort, L., Rotllant, J., Rovira, L., 1998. Immunological suppression in gilthead sea bream *Sparus aurata* of the
1182 North-West Mediterranean at low temperatures. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.*
1183 120, 175–179. [https://doi.org/10.1016/S1095-6433\(98\)10027-2](https://doi.org/10.1016/S1095-6433(98)10027-2)
- 1184 Trinchera, L., Russolillo, G., 2010. On the use of Structural Equation Models and PLS Path Modeling to build
1185 composite indicators. WP n.22 DiSSE, Univ. Macerata, Italy. 44.
- 1186 Uluşu, N.N., Acan, N.L., Turan, B., Tezcan, E.F., 2003. Inhibition of glutathione reductase by cadmium ion in
1187 some rabbit tissues and the protective role of dietary selenium. *Biol. Trace Elem. Res.* 91, 151–156.
1188 <https://doi.org/10.1385/BTER:91:2:151>
- 1189 Valerie, D., Antonin, G., Dominique, M., Vincent, O., Gregory, M., Martine, F., Samuel, P., Serge, M., Jean-
1190 Louis, G., Melanie, B., Charlotte, P., Elise, H., Béatrice, B., Nathalie, M., Annie, F., 2017. OBSLAG
1191 2016 - volet eutrophisation. Etat DCE des lagunes méditerranéennes (eau et phytoplancton, période 2011-
1192 2016). Développement d'indicateurs de tendance et de variabilité. RST-LER/LR 17.10.
- 1193 Valko, M., Morris, H., Cronin, M., 2005. . Metals, Toxicity and Oxidative Stress. *Curr. Med. Chem.* 12, 1161–
1194 1208. <https://doi.org/10.2174/0929867053764635>
- 1195 Van Anholt, R.D., 2004. Arachidonic acid reduces the stress response of gilthead seabream *Sparus aurata* L. *J.*
1196 *Exp. Biol.* 207, 3419–3430. <https://doi.org/10.1242/jeb.01166>
- 1197 Vera, L.M., Montoya, A., Pujante, I.M., Pérez-Sánchez, J., Calduch-Giner, J.A., Mancera, J.M., Moliner, J.,
1198 Sánchez-Vázquez, F.J., 2014. Acute stress response in gilthead sea bream (*Sparus aurata* L.) is time-of-
1199 day dependent: Physiological and oxidative stress indicators. *Chronobiol. Int.* 31, 1051–1061.
1200 <https://doi.org/10.3109/07420528.2014.945646>
- 1201 Vinzi, V.E., Trinchera, L., Amato, S., 2010. Handbook of Partial Least Squares. <https://doi.org/10.1007/978-3-540-32827-8>

1203 Volkman, J.K., Johns, R.B., Gillan, F.T., Perry, G.J., Bavor, H.J., 1980. Microbial lipids of an intertidal
1204 sediment. Fatty acids and hydrocarbons. *Geochim. Cosmochim. Acta* 44, 1133–1143.
1205 [https://doi.org/10.1016/0016-7037\(80\)90067-8](https://doi.org/10.1016/0016-7037(80)90067-8)

1206 Wajsbrov, N., Gasithb, A., Kromc, M.D., Poppep, D.M., 1991. Acute toxicity of ammonia to juvenile gilthead
1207 seabream *Sparus aurata* under reduced oxygen levels. *Aquaculture* 92, 277–288.
1208 <https://doi.org/10.1515/9783110528480-008>

1209 Werner, E.E., Gilliam, J.F., Hall, D.J., Mittelbach, G.G., 1983. An experimental test of the effects of predation
1210 risk on habitat use in fish. *Ecology* 64, 1540–1548. <https://doi.org/10.2307/1937508>

1211 Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A., Langham, G., 2008. Towards an integrated framework
1212 for assessing the vulnerability of species to climate change. *PLoS Biol.* 6, e325.
1213 <https://doi.org/10.1371/journal.pbio.0060325>

1214 Wilson, A.J., Nussey, D.H., 2010. What is individual quality? An evolutionary perspective. *Trends Ecol. Evol.*
1215 25, 207–214. <https://doi.org/10.1016/j.tree.2009.10.002>

1216 Wingfield, J.C., Romero, L.M., 2011. Adrenocortical Responses to Stress and Their Modulation in Free-Living
1217 Vertebrates, in: *Comprehensive Physiology*. John Wiley & Sons, Inc., Hoboken, NJ, USA.
1218 <https://doi.org/10.1002/cphy.cp070411>

1219 Wright, D.A., 1995. Trace metal and major ion interactions in aquatic animals. *Mar. Pollut. Bull.* 31, 8–18.
1220 [https://doi.org/10.1016/0025-326X\(95\)00036-M](https://doi.org/10.1016/0025-326X(95)00036-M)

1221 Zhao, S., Feng, C., Wang, D., Liu, Y., Shen, Z., 2013. Salinity increases the mobility of Cd, Cu, Mn, and Pb in
1222 the sediments of Yangtze Estuary: Relative role of sediments' properties and metal speciation.
1223 *Chemosphere* 91, 977–984. <https://doi.org/10.1016/j.chemosphere.2013.02.001>
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1228 **FIGURE CAPTIONS**

1229 **Figure 1. Path diagram of the structural model.**

1230 **Figure 2. Map of the sampling area. Each sampling site is identified by its local French**
1231 **name.**

1232 **Figure 3. Partial Least Square (PLS) path model showing the strength and direction of**
1233 **the relationships among Latent variables. Red represents negative direct effects while**
1234 **positive relationships are represented in green. The thickness of the arrows is**
1235 **proportional to the strength of the effect and dashed arrows represent non-significant**
1236 **relationships ($p_{\text{value}} > 0.05$). Direct effects are shown as standardized path coefficients.**
1237 **Loadings of each Measured Variable (MV) in relation to its Latent Variable (LV) are**
1238 **shown for each block in its respective frame.**

1239 **Figure 4. Summary of direct and indirect effects of independent latent variables (LVs)**
1240 **on fish stress. The total effect of an independent LV on its dependent LV is the sum of**
1241 **all indirect effects and the direct effect.**

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1243 **TABLE CAPTIONS**

1244 **Table 1. Fish sampling summary table. Descriptive statistics are given for each lagoon in**
1245 **terms of total length, mass, age estimated by scale reading, and growth rate centred by**
1246 **age. We gathered all information required for the present study for 96 of the 100**
1247 **sampled fish. For four individuals, one or two assays were lacking due to limited blood**
1248 **material and they were removed from the analysis.**

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1250 **Table 2. Summary of the different trophic and diet quality markers selected for the**
1251 **analysis and associated references. Based on Blanchet-Aurigny et al. 2015. For an**
1252 **exhaustive review, see Pernet (2016).**

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1254 **Table 3. Models comparisons. GOF Goodness-of-fit statistics, calculated as the**
1255 **geometric mean of the average block communality and the average R^2 value, thus**
1256 **accounting for the quality of both the measurement and the structural models. The**
1257 **quality of the manifest model is evaluated for each latent variable (LV) by block**
1258 **communality, which is calculated as the average of all squared correlations between the**
1259 **given LV and its underlying manifest variables (MVs). Block communality thus**
1260 **measures how much of the variance is common between a LV and its MVs (Vinzi et al.,**
1261 **2010). Finally, the fit of the structural model is assessed for each endogenous LV (R^2),**
1262 **which highlights the amount of variance in the endogenous LV explained by its**
1263 **independent LVs (Vinzi et al., 2010).**

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1265 **Table 4. Summary of the different parameters measured in fish, the tissue considered,**
1266 **their unit, their role as biomarkers and the latent variable they refer to.**

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1268 **Table 5. Summary of the model 7; Exogenous refers to Latent Variable in the model for**
1269 **which no incoming effect are tested (no arrows pointed at them; lagoon features and**
1270 **age).**

1271 **Table 6. Summary of the latent model. Effect size of the relationships between each LVs**
1272 **within the model 7 are given (estimates) as well as their error (standard deviation) and t-**
1273 **values. *Pvalues are considered when $\alpha < 0.05$.**

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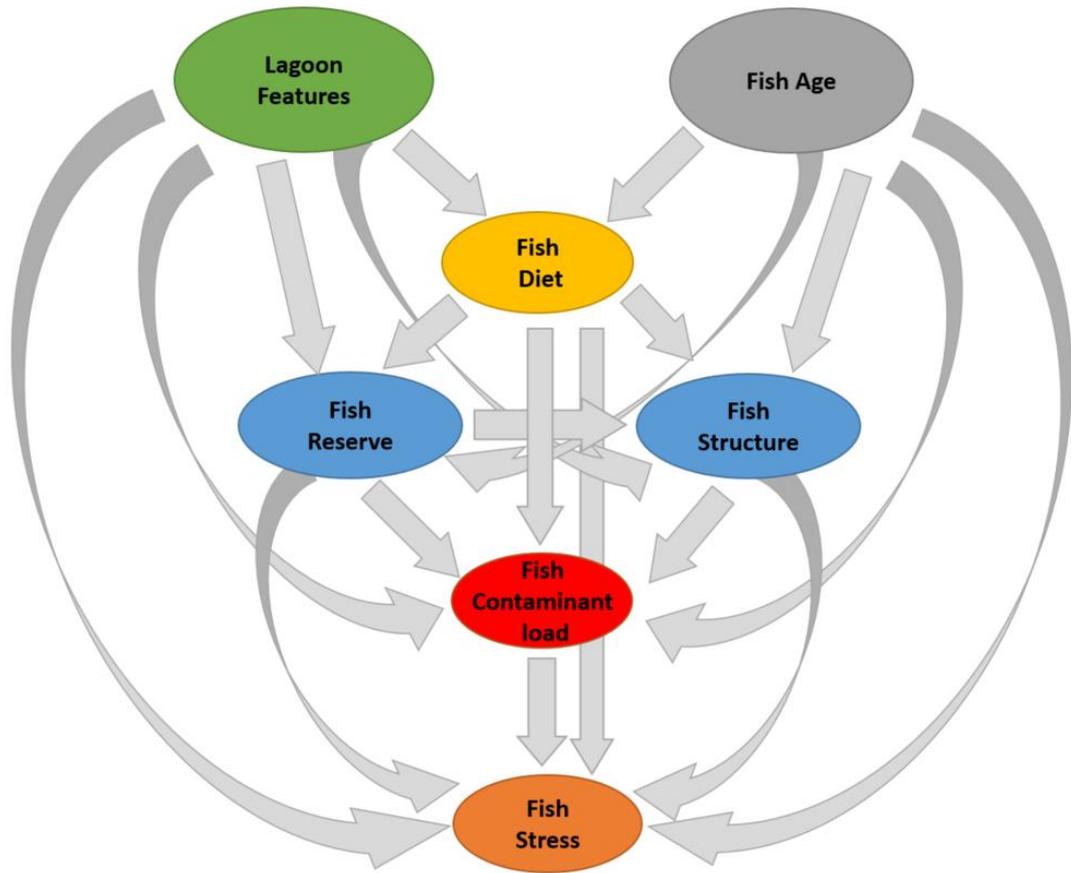
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1290 **FIGURES**

1291 **Figure 1.**



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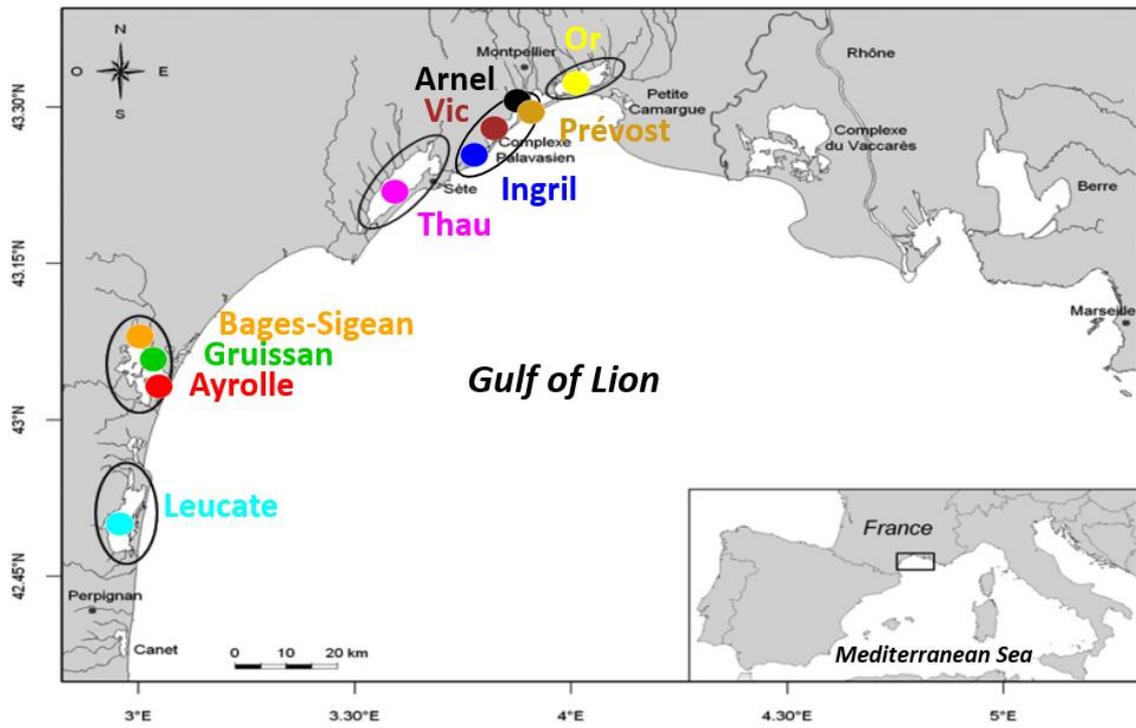
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1301 **Figure 2.**



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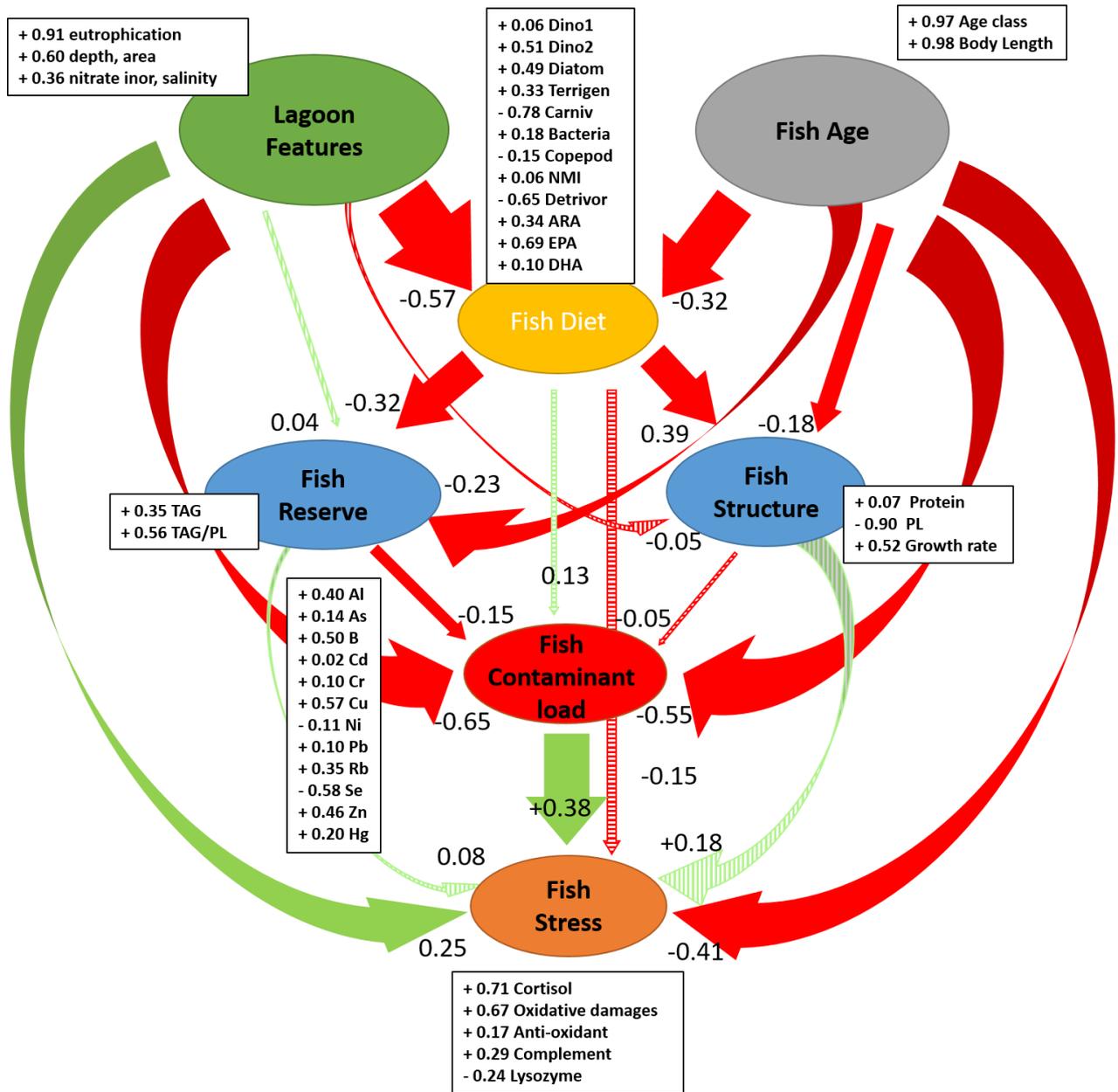
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1313 **Figure 3.**



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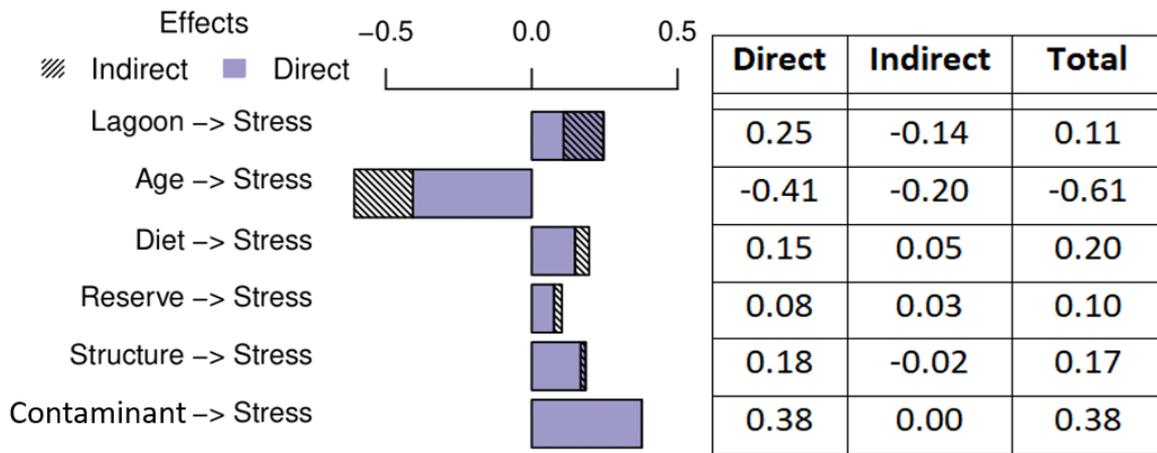
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1321 **Figure 4.**



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1335 **TABLES**

1336 **Table 1.**

		Total Length		Mass		Age		Growth rate	
		(mm)		(g)				(centred by age)	
Lagoon	n	Mean \pm sd	min-max	Mean \pm sd	min-max	Mean \pm sd	min-max	Mean \pm sd	min - max
Arnel	10	190.6 \pm 4.7	185 - 200	97 \pm 6	89 - 108	0.0 \pm 0.0	0-0	0.17 \pm 0.18	-0.07 - 0.5
Ayrolle	10	281.3 \pm 17.9	254 - 300	331 \pm 65	238 - 430	1.6 \pm 0.5	1-2	-0.01 \pm 0.47	-0.77 - 0.6
Gruissan	9	299.1 \pm 57.2	158 - 324	429 \pm 153	52 - 516	1.8 \pm 0.7	0-2	1.09 \pm 1.00	-1.32 - 1.69
Ingril	10	274.5 \pm 28.2	232 - 313	305 \pm 104	160 - 436	1.7 \pm 0.5	1-2	-0.27 \pm 1.11	-1.8 - 1.15
Leucate	9	277.0 \pm 9.4	265 - 295	293 \pm 32	248 - 354	1.8 \pm 0.4	1-2	-0.37 \pm 0.43	-1.05 - 0.32
Or	9	201.6 \pm 8.3	191 - 214	139 \pm 22	116 - 188	0.0 \pm 0.0	0-0	1.01 \pm 0.38	0.45 - 1.75
Prévost	10	261.4 \pm 48.9	132 - 305	282 \pm 90	174 - 408	1.5 \pm 0.5	1-2	-0.35 \pm 0.98	-2.01 - 0.85
Bages-Sigean	9	280.1 \pm 9.6	265 - 295	324 \pm 32	274 - 361	1.0 \pm 0.0	1-1	1.04 \pm 0.55	0.19 - 1.83
Thau	10	264.7 \pm 11.1	249 - 286	267 \pm 37	218 - 333	2.0 \pm 0.0	2-2	-1.09 \pm 0.58	-1.89 - 0.02
Vic	10	190.8 \pm 30.5	106 - 209	108 \pm 9	97 - 125	0.0 \pm 0.0	0-0	0.56 \pm 0.28	0.09 - 0.92

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1346 **Table 2.**

Marker	Source	Reference
Bacteria: iso15:0 + ant15:0 + 15:0 + iso16:0 + iso 17:0 + 17:0	Bacteria	(Volkman et al., 1980)
Dino1: 16:1 ω 7/16:0	Dinoflagellates	(St John and Lund, 1996)
Dino2: 20:5 ω 3/22:6 ω 3	Diatoms vs dinoflagellates	(Budge and Parrish, 1998)
Diatom : 16:1 ω 7/20:5 ω 3	Diatoms	(Pernet, 2016)
Detrital: SFA/PUFA	Phytoplankton vs detrital	(Biandolino et al., 2008; Maazouzi et al., 2007; Pommier et al., 2010; Prato et al., 2012)
Copepods: 20:1 ω 9 + 22:1 ω 11	Copepods	(Budge et al., 2002; Kattner et al., 2012)
Terrigenous: 18.2 ω 6 + 18.3 ω 3	Terrestrial vascular plants, Green microalgae / Terrigenous	(Budge and Parrish, 1998; Dalsgaard et al., 2003; Kelly and Scheibling, 2012)
non-methylene interrupted (NMI) 20:2i + 20:2j + 22:2i + 22:2j	Bivalves and gastropods	(Budge et al., 2007; Joseph, 1982)
Carnivorous: 18:1 ω 9/18:1 ω 7	Carnivorous	(Auel et al., 2002; El-Sabaawi et al., 2009; Graeve et al., 1997)
Essential FA	Role	Reference
Docosahexaenoic acid (DHA): 22:6 ω 3	Essential FFA: neuronal development and maintenance	(Arts and Kohler, 2009; Bell and Sargent, 2003, 1996; Bell et al., 1995; Koussoroplis et al., 2011; Koven et al., 2003; Schmitz and Ecker, 2008; Van Anholt, 2004)
Arachidonic acid (ARA): 20:4 ω 6	Essential FFA: precursor of eicosanoid hormone	
Eicosapentaenoic acid (EPA): 20:5 ω 3	Essential FFA: precursor of eicosanoid hormone	

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1355 **Table 3.**

Model	Model specificity	Lvs	Mvs	Direct Paths	GOF	Lagoon		Diet		Reserve		Structure		Contaminant		Stress		Age	
						Block	Block	R ²	Block										
1	Basic	7	38	19	0.2908	0.472	0.191	0.42	0.106	0.19	0.360	0.26	0.132	0.638	0.206	0.544	1.000		
7	Basic + effect of Lagoon on Age	7	38	21	0.2733	0.391	0.195	0.317	0.127	0.187	0.362	0.303	0.126	0.587	0.203	0.546	1.000	0.305	
2	Basic with Size instead Age	7	38	20	0.283	0.433	0.192	0.332	0.123	0.141	0.358	0.316	0.120	0.613	0.223	0.577	1.000		
3	Basic with Size + Age	7	39	19	0.3091	0.436	0.197	0.283	0.141	0.156	0.361	0.355	0.123	0.631	0.223	0.535	0.940		
4	Basic + effect of Structure on Reserve	7	38	20	0.2972	0.459	0.195	0.254	0.212	0.401	0.227	0.215	0.130	0.634	0.227	0.525	1.000		
5	Basic + effect of Reserve on Structure	7	38	20	0.3074	0.478	0.185	0.414	0.193	0.195	0.359	0.468	0.133	0.63	0.207	0.548	1.000		
6	Basic + effect of Reserve on Structure with Size + Age	7	39	20	0.3262	0.439	0.193	0.283	0.212	0.160	0.359	0.550	0.124	0.626	0.225	0.538	0.943		

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1371 **Table 4.**

Parameters	Tissue	Unit	Role	Latent Variable
Cortisol	Scales	$\mu\text{g.g}^{-1}$	Systemic stress hormone	Fish Stress
Oxidative damages	Plasma	$\text{mg H}_2\text{O}_2$ equivalent. dL^{-1}	Oxidative damages accumulated	Fish Stress
Antioxidant	Plasma	$\mu\text{mol HCl.mL}^{-1}$	Oxidative defences available	Fish Stress
Complement	Plasma	%lysis	Efficiency of the innate immune function (complement pathway)	Fish Stress
Lysozyme	Plasma	U.mL^{-1}	Efficiency of the innate immune function (Lysozyme enzymatic efficiency)	Fish Stress
Inorganic contaminants	Muscle	$\mu\text{g.g}^{-1}$ dry muscle	Contamination load by each inorganic contaminants	Fish contaminant
Protein	Muscle	mg.g^{-1} dry muscle	Body condition index – quantity of proteins allocated in structure	Fish Structure
Lipids	Muscle	mg.g^{-1} dry muscle	Body condition index - Types and quantity of lipids stored in the muscle	Fish Reserve & Fish Structure
Fatty acids	Muscle	% total - ratios	Trophic markers - Types and ratios of fatty acids present in the muscle	Fish Diet

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1383 **Table 5.**

<i>LATENT VARIABLE</i>	Type	Manifest variables	R ²	Block Communality
<i>Lagoon features</i>	Exogenous	↑ Eutrophication, depth & area and dissolved inorganic nitrogen ↓ Salinity		0.439
<i>Fish Age</i>	Exogenous	↑ Age ↑ Fish total length		0.943
<i>Fish Diet</i>	Endogenous	↑ Carnivorous, Copepods ↓ Dinoflagellates, Diatoms 1 & 2, Terrigenous, Bacteria NMI, Detrital, ARA, EPA, DHA	0.283	0.193
<i>Fish Reserve</i>	Endogenous	↑ TAG and TAG/PL	0.160	0.212
<i>Fish Structure</i>	Endogenous	↑ Growth rate ↓ PL	0.550	0.359
<i>Fish contaminant load</i>	Endogenous	↑ Al, As, B, Cr, Cu, Rb, Zn, Hg ↓ Se, Ni r≤0.10 Cd, Cr, Pb	0.626	0.124
<i>Fish Stress</i>	Endogenous	↑Cortisol, oxidative stress damages, anti-oxidant, complement activity ↓Lysozyme activity	0.538	0.225
<i>Goodness of fit</i>	0.3262			

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1392 **Table 6.**

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	Estimate	Std. error	t value	P value
\$ Fish Diet				
<i>Intercept</i>	0.000	0.088	0.000	1
<i>Lagoon features</i>	0.571	0.096	5.960	4.48e-8*
<i>Fish Age</i>	0.321	0.096	3.650	1.15e-03*
\$ Fish Reserve				
<i>Intercept</i>	0.000	0.096	0.000	1
<i>Lagoon features</i>	0.037	0.123	0.302	0.763
<i>Fish Age</i>	-0.231	0.110	-2.100	0.039*
<i>Fish Diet</i>	0.318	0.113	2.820	0.006*
\$ Fish Structure				
<i>Intercept</i>	0.000	0.070	0.000	1
<i>Lagoon features</i>	-0.048	0.090	-0.329	0.596
<i>Fish Age</i>	-0.183	0.083	-4.370	0.030 *
<i>Fish Diet</i>	0.391	0.087	3.680	1.84e-05 *
<i>Reserve</i>	0.499	0.077	6.500	4.28e-09
\$ Fish contaminant load				
<i>Intercept</i>	0.000	0.064	0.000	1
<i>Lagoon features</i>	0.650	0.083	-7.840	8.56e-12*
<i>Fish Age</i>	-0.555	0.078	-7.090	2.94e-10*
<i>Fish Diet</i>	-0.132	0.088	-1.510	0.135
<i>Fish Reserve</i>	-0.149	0.085	-1.750	0.084
<i>Fish Structure</i>	-0.047	0.096	-0.485	0.629
\$ Fish Stress				
<i>Intercept</i>	0.000	0.072	0.000	1
<i>Lagoon features</i>	0.246	0.120	2.050	0.043*
<i>Fish Age</i>	-0.408	0.109	-3.730	3.3e-4*
<i>Fish Diet</i>	0.149	0.099	1.500	0.138
<i>Fish Reserve</i>	0.076	0.097	0.790	0.432
<i>Fish Structure</i>	0.185	0.107	1.720	0.089*

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<i>Fish contaminant load</i>	0.378	0.118	3.210	0.002*
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