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An integrative perspective on fish health: Environmental and anthropogenic pathways affecting fish stress

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Abstract :

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

Highlights

► Assessing cumulative effects of natural & anthropogenic stressors on individual stress response. ► To understand how organisms cope with environmental change and contaminants. ► Ten environmental variables and 36 physiological variables into one PLS-PM model. ► 54 % of fish stress explained, notably by inorganic contaminant load.

Keywords : Ecosystem, PLS-PM, Eco-physiology, Direct and indirect effects, Fish health, Stress

INTRODUCTION

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

Such approaches should be particularly useful in transitional ecosystems, such as coastal lagoons, where organisms are subject to a high diversity of environmental pressures. Together with estuaries, coastal lagoons form part of a continuum between continental and aquatic ecosystems and are consequently exposed to highly variable hydrological conditions (Pérez-Ruzafa et al., 2011). Situated at the interface between open sea and land, these areas of high biological productivity receive high inputs of organic matter and nutrients of natural and human

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

Stress as an integrative measure of individual health

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

overwhelmed by environmental stressors (Boonstra, 2013; Boonstra et al., 2007; MacDougall-Shackleton et al., 2019).

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

Factors affecting individual health and the case of aquatic ecosystems

Extrinsic (e.g. predation, parasitism, microclimate, pollution, etc.) and intrinsic (e.g. individual age, body condition, life history stage, etc.) factors may affect organism stress through both direct and indirect pathways. In aquatic ecosystems, limited oxygen (Wajsbrov et al., 1991), extreme osmotic (Laiz-Carrión et al., 2005; Tandler et al., 1995) or temperature (Heather et al., 2018) conditions have direct impacts on organism's immune function (Cuesta et al., 2005) and oxidative status (Madeira et al., 2016, 2013). Metal contaminants accumulated in an organism also have direct long-term consequences on anti-oxidant depletion (Médale et al., 2005; Saera-Vila et al., 2009), enhancing oxidative stress (Benhamed et al., 2016; Mourente et al., 2002; Soares et al., 2008) and impairing immune function (Cerezuela et al., 2016;

Guardiola et al., 2016, 2015). Similarly, individual age or experience also directly affects the way organisms cope with given stressors; older individuals often coping more efficiently with stressors owing to past experience, until late life senescence (Barcellos et al. 2012, Navaratnarajah and Jackson 2013). For instance, age- and species-specific changes in bio-accumulation or sensitivity to specific pollutants are known to evolve: metal contaminants such as mercury (Hg) appear to bio-accumulate over life (Ourgaud et al., 2018), whereas others are detoxified or diluted through time (Lee et al., 1998).

Besides these direct effects, extrinsic and intrinsic factors also influence individual stress through indirect pathways. In particular, environment-driven changes (e.g. temperature, light, salinity, pH) affecting lower trophic levels of the food web, can also affect higher trophic levels indirectly by modifying prey availability (Chassot et al., 2010). Assessing variation in individual diets may therefore present a convenient tool for tracking indirect effects on individual energy stored, growth and stress. Organic matter inputs are central to aquatic ecosystem functioning, affecting primary production (phytoplankton vs. aquatic angiosperms in eutrophic vs. oligotrophic conditions), as well as higher trophic levels (i.e. affecting the nature of reserves fish assimilate as well as their growth capacities; Escalas et al., 2015; Isnard et al., 2015; Le Fur et al., 2019). For instance, whereas fish grow faster at intermediate salinities, salinity also has complex interactions with temperature and oxygen concentrations, and the consequences on food intake and food conversion are species-dependant (Bœuf and Payan, 2001). Increasing salinity also limits metal contaminant accessibility in animal (Ifremer, 2009; Lee et al., 1998) and bioaccumulation most probably due to osmo-regulation modulation (Lee et al., 1998; Wright, 1995) but biodisponibility in the water and sediments tend to improve (Zhao et al., 2013).

Study aim and choice of a relevant fish model

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

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

We expected pollutant loads to directly impair individual health. We further expected abiotic and biotic environmental features (eutrophication, organic matter, salinity and depth), as well as individual age to affect seabream growth and condition, through changes in their diet. Since fish condition is known to interact with pollutant bio-accumulation, and therefore

indirectly with stress, the use of a PLS-PM approach (see Fig. 1 for the general construct) and comparison of seven alternative models, allowed for a comprehensive study into how environmental conditions, individual condition and environmental pollution affected fish health in coastal lagoons.

METHODS

Environmental descriptors

We monitored ten coastal lagoons (Arnel, Ayrolle, Gruissan, Ingril, Leucate, Or, Prévost, Bages-Sigean, Thau, and Vic; latitude: from 48° 51' 18" N to 43° 34' 50" N; longitude from 3° 0' 21" E to 4° 2' 16" E; see Fig. 2) in the Gulf of Lions (France, NW Mediterranean Sea, Fig. 2). Lagoon morphological features included surface area (km^2), maximum depth (m), and hydrobiological characteristics of the water column (collected 1 m below the surface), including salinity, dissolved inorganic nitrogen (DIN, µM), total nitrogen (µM), total phosphorus (µM), phosphate (µM), chlorophyll *a* ($\mu g.L^{-1}$), dissolved oxygen concentration (O_2, $mg.L^{-1}$), and Turbidity (NTU), as integrative descriptors of the environment at a lagoon scale (ESM1). Those data were acquired via the OBSLAG network and the Water Framework Directive (WFD) (Bouchoucha et al., 2019; Derolez et al., 2017). Data were collected monthly from 2014-17 (time period corresponding to the age in years of the sampled seabreams, see below) during summer (June, July, August), from a single station in smaller lagoons (Arnel, Ayrolle, Gruissan, Ingril, Prévost, and Vic), two stations in intermediate sized lagoons (Bages-Sigean, Leucate and Or), and six stations in the largest lagoon (Thau).

Linking lagoon morphometric and hydrobiological features to relevant biological functions or mechanisms is not trivial and previous studies have addressed this issue (see Derolez et al., 2020 and references therein). Following recommendations, we partitioned

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

Fish sampling

Seabream were sampled from the same ten coastal lagoons in autumn 2017. Fish were sampled just before their annual winter migration from the lagoons. Individuals were fished using traditional Mediterranean fishing gear ("capetchades") from October 10th to 30th 2017. This fishing method uses a thin mesh to capture and sample live fish. The device was deployed in the evening; fish entered during the night and were collected early the next morning. We captured over 100 individuals, focusing on the smallest fish, to insure all were young individuals and incidentally males (as seabreams are proteandrous hermaphrodites). Fish were kept in oxygenated lagoon-water tanks until sacrificed by immersion in ice-cooled lagoon-water tanks and a blood sample collected within 1 hour of capture. We collected 0.5 mL of blood from the caudal vein using a 25G heparinised syringe. After centrifugation (3500g for 10 min) plasma was separated and kept frozen at −80°C until analysed. Individuals were weighed to the

nearest ± 1g and their total length measured to the nearest ± 1mm using a metal ruler (Table 1). Fish were kept on crushed ice for transport to the lab, then frozen at -20°C until analyses. Four weeks later, all individuals were unfrozen and we collected the scales over the entire right flank of the fish flank for stress hormone (cortisol) content measures, and 20 to 30 scales below the pectoral fin on the opposite flank to confirm individual age from scale growth annuli. Three pieces of dorsal epaxial muscle (white muscle) were saved and kept frozen at -80°C for pollutant, lipid and protein content analyses. Muscle weight was measured before and after lyophilisation to account for individual hydration level. Measurements on muscle (pollutants, lipid, FA) are reported relative to muscle dry weight.

Individual age and growth rate

Individual age (in years) was determined by counting annual increments on five to seven scales immersed in aqueous solution using transmitted light, as routinely done in seabream (for details see Mahé et al., 2009).

We estimated individual growth rates by dividing individual total length by age. As growth rate drastically decreases after the first year and to account for variation in growth rate between ages, we standardized growth rate by age class (1, 2, 3). A summary of sampled fish per lagoon is given Table 1.

Body composition

Lipid content

Lipids were extracted from fish muscle following Folch et al. (1957) and lipid classes separated and quantified using an Iatroscan MK-VI (Iatron Laboratories, Inc.) following Parrish (1999) (for details; see Sardenne et al., 2019). Lipids were broadly classified into reserve (e.g. fat

stores) or structural (e.g. membrane phospholipids) lipids. Reserve lipids are constituted of triacylglycerols (TAG), diacylglycerols (DAG as precursors of TAG) and free fatty acids (FFA). In contrast, structural lipids include phospholipids (PL) and sterols (ST). Finally, acetone-mobile polar lipids (AMPL), and alcohols (ALC) are eluted at the same time but present in relative low proportions (Tocher, 2003). PL represented the predominant lipid (ca. 87%; 45 to 98% of total lipids, TL) in our samples, whereas TAG concentration showed important inter-individual variation (1 to 51 % of TL). FFA, ALC, DAG and AMPL were very low (<0.05%) or undetected, while ST were always detected at low level (< 2% of TL, see ESM2). Low FFA proportion indicated that lipid integrity was conserved during sample storage (Parrish, 1999).

Muscle Protein content

Protein content was assessed using a piece of epaxial muscle after lyophilization and grinding (ball mill, MM400, Retsch GmbH, Germany). Proteins were extracted from 10 mg of dry muscle powder by immersion and incubation in a 1.5-mL solution of a 10% SDS (Sigma Aldrich), 1.5% Protease inhibitor Cocktail (cOmplete, Sigma Aldrich, France), miliQ water solution (lysis solution adapted from Campus et al. 2010). Cycles of successive 15-min ultrasonic baths (300 Ultrasonik, Ney Company, USA) and 3-min vortex were repeated four times to allow total dissolution of the sample. Extracts were clarified by a 10-min centrifugation at 3000g at 4°C (Ericsson and Nistér, 2011). Muscle protein content in 25 µL of the collected fraction was quantified using a Bicinchoninic Acid method (BCA, Pierce, Thermo Fisher Scientific, France). Intra- and inter-plate protein variations (based on the same sample repeated over plates) were 7.62% and 16.7%, respectively.

Choice of body composition indices

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

Trophic markers and Fatty Acid ratio indicators

Fatty acid identification

The polar fraction of lipids, mainly PL, plays an important role in cell structure and shows a high degree of plasticity in response to changes in abiotic environmental features. The neutral fraction plays a role as a resource compartment, destined either for oxidation to provide energy (ATP) or for incorporation into PL. Predominantly made out of TAG and wax esters, its composition is less influenced by changes in abiotic features and therefore better reflects individual diet (reviewed in Dalsgaard et al., 2003). Therefore, only the neutral fraction was considered in this study. Neutral and polar lipids were separated by column chromatography on silica gel and methylated using 12% BF_3-MeOH (see ESM3). Fatty acid methyl esters (FAMEs) were analysed in an Agilent 6890 gas chromatograph equipped with an on-column injector and a flame-ionization detector, with hydrogen as a carrier gas. They were identified by their retention times with reference to those of a standard 37-component fatty acid methyl esters (FAME) mix and designated following the formula C:X(n-Y) where C is the number of carbon atoms, X is the number of double bonds and Y is the position of the first double bond counted from the CH terminal.

Choice of diet indicators

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

Metal pollutant load in fish muscle

Muscle samples were treated with trace mineral grade nitric acid (69% Suprapure, Merck) and 33% H_2O_2 (Suprapure, Merck) in Teflon reaction tubes, heated in a microwave digestion system (UltraClave-Microwave Milestone®) for 20 min at 220 °C, and then diluted to 10 mL with double-deionized water (MilliQ).

Total mercury (Hg) content was measured using an atomic absorption spectrometer AMA254 Advanced Mercury Analyzer (Leco) (Bouchoucha et al., 2018), without pre-treating or pre-concentrating the samples (wavelength = 253.65 nm, detection limit (DL) = 0.003 µg g^{-1}).

Samples were further analysed using inductively coupled plasma optical emission spectrometry (ICP-OES, ICAP 6500 Duo, Thermo) to quantify levels of aluminium (Al), arsenic (As), boron (B), cadmium (Cd), chrome (Cr), copper (Cu), nickel (Ni), lead (Pb), rubidium (Rb), Selenium (Se) and zinc (Zn) (García-Navarro et al., 2017). The DL for the analysed elements was 0.001 µg.g^{-1}. Each sample was read in duplicate and averaged. Based

on UNE-EN ISO reference 11885, multi-element calibration standards (SCP Science, in 4% nitric acid) were assembled with different concentrations of inorganic elements.

Individual stress status

Stress hormone: scale cortisol content

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

Fish oxidative status

We assessed global measures of oxidative damages and antioxidant defences in plasma using the d-ROMs test and OXY Adsorbent tests (Diacron International©, Grosseto, Italy). Oxidative damage was assessed in 8 µL of plasma by measuring hydroperoxides, the main compounds contributing to plasma oxidant activity, expressed as mg H_2O_2 equivalent/dL (Beauvieux et al., 2022). Oxidative defences were evaluated in 5 µL of 1:100 diluted plasma by measuring the ability of plasma to buffer massive oxidation through hydroperoxide acid, expressed in µmol HCl/mL (Beauvieux et al., 2022). All sample measurements were run in duplicate and averaged. Intra-plate variations were 2.40% and 7.51% for ROMs and OXY, respectively. Inter-plate variations were 10.02% and 7.92% for ROMs and OXY, respectively.

Immune functions: Natural haemolytic complement and lysozyme activities

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

haemolysis obtained from SRBC without serum. The power of haemolysis (%) was estimated as the slope of its lysis curve (Díaz-Rosales et al., 2006). Intra- and inter-plate variations were 9.06% and 14.71%, respectively.

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

Statistics

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

We investigated the multi-scale causal pathways by which extrinsic (environmental and anthropogenic) and intrinsic factors affected seabream ecophysiology using Partial Least

394 Squares Path Modelling (PLS-PM) (R package plspm, Sanchez 2013). PLS-PM allows studying
395 complex multivariate relationships among groups of manifest (observed) and latent (construct)
396 variables (MVs and LVs respectively), testing for direct and indirect effects. These effects are
397 necessarily unidirectional (Sanchez, 2013; Vinzi et al., 2010) and can be used to draw causal
398 inferences in the path diagram (more detailed framework presented in ESM4). LVs are
399 hypothetical constructs that are not measured directly but can be assumed to reflect their
400 underlying MVs. The general construct of this study is detailed in Fig. 1.

401 Using a PLS-PM approach, we defined extrinsic and intrinsic constructs (LVs), built in
402 a formative way (for detail see ESM4), characterizing relevant environmental and physiological
403 pressures affecting seabreams in coastal lagoons. We then explored how individual stress was
404 related to those morphological and hydrobiological, anthropogenic (pollutants), dietary and
405 intrinsic constructs using an iterative algorithm (Trinchera and Russolillo, 2010), and
406 comparing seven alternative models (Table 3). The 7 constructs (LVs) always included: (1)
407 lagoon features (morphological and hydrobiological); and fish (2) diet, (3) pollutant loads, (4)
408 endogenous reserves, (5) structure components, (6) age, and (7) stress. Each of these latent
409 variables was the combination of one or more MVs (such as lagoon eutrophication, lagoon
410 depth, fish cortisol levels, etc.). Expected directional relationships were based on empirical and
411 theoretical research to draw a comprehensive picture of the complex ecosystem interactions
412 likely affecting seabream/fish stress in coastal lagoons, and alternatives were assessed for seven
413 different causal models (see ESM5). We tested for potential effects of lagoon features on fish
414 age (Model 2), and tested if the LV fish age was best predicted by structural size, age in years
415 or both (Model 1, 3 & 4). Models 1, 5 & 6, differed in terms of directional relationships (e.g.
416 fish reserve affected fish structure, the opposite, or no relation was specified between those
417 LVs; Table 3). We computed a final model including all of the interactions retained in previous
418 models (Model 7). For each model, we computed and compared the overall variance explained

by the path model (goodness of fit, GoF) (Table 3, ESM5). The model displaying the highest GoF was retained and discussed in the manuscript.

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

RESULTS

Model Comparisons

The seven models displayed similar global prediction performances (GoF) ranging from 27.3% to 32.6% (Table 3). Regardless of the model considered the relationships between connected latent variables (LVs) that were significant remained identical in terms of sign (positive or negative; ESM5) and only differed slightly in terms of strengths. Model 7 displayed the highest GoF (32.6 %) and therefore was selected for further investigation.

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

Manifest model variance

442 The variance of latent variables (LVs) explained by manifest variables (MVs) is given by the
443 block communality coefficients (Table 5). For instance, 44% of the variance in data was shared
444 between the LV "lagoon features" and its three MVs, *i.e*. the three principal components of the
445 PCA representing eutrophication, lagoon morphology (depth and area), and inorganic nitrogen
446 (DIN) and salinity variables (Table 5). The LV "fish age" included the age and size of fish and
447 accounted for 94% of the observed variation. Other LVs shared a lesser proportion of variance
448 with their underlying MVs, ranging from 12 to 36%. Table 5 summarizes all explained variance
449 from LVs and Fig. 3 describes how they related to the underlying (measured) MVs. First, the
450 LV "lagoon features" was positively related to eutrophication (r = 0.91), lagoon depth and area
451 (r = 0.60) and the third PCA axis encompassing increasing DIN and decreasing salinity (r
452 = 0.36). Second, the LV "fish diet" was negatively related to dinoflagellates 2 (r = -0.51),
453 diatoms (r = -0.49), terrigenous inputs (r = -0.33), bacteria (r = -0.18), detrital sources (r = -
454 0.65), and arachidonic acid (ARA) (r = -0.34) and eicosapentaenoic acid (EPA) (r = -0.69) as
455 essential free FA sources, while positively related to carnivorous (r = 0.78) and copepods (r =
456 0.15) inputs. DHA (r = 0.10), dinoflagellates 1 (r = -0.06), and non-methylene interrupted
457 (NMI) (r = -0.06) contributions to the LV "fish diet" remained small ($r \leq 0.10$). Third, the LV
458 "fish reserve" corresponded to an increase in TAG (r = 0.35) and TAG/PL ratio (r = 0.56), both
459 being proxies of fish body condition. Fourth, the LV "fish structure" positively correlated to
460 growth rate (r = 0.52) and negatively correlated (r = -0.90) with the amount of phospholipid
461 present in the neutral FA stored. Muscle protein concentration only marginally contributed to
462 the LV "Fish structure" (r = 0.07). Fifth, the LV "fish pollutant" load positively correlated to
463 metal concentrations of Al (r = 0.40), As (r = 0.14), B (r = 0.50), Cu (r = 0.57), Rb (r = 0.35),
464 Zn (r = 0.46), Hg (r = 0.20) and negatively correlated to concentrations of Se (r = -0.51) and Ni
465 (r = -0.11). Cd, Cr, and Pb contributions were marginal ($r \leq 0.10$). Finally, the LV "Fish stress"
466 highly and positively correlated to cortisol (r = 0.71), oxidative damage (ROM, r = 0.67),

complement (r = 0.29), and antioxidant defences (OXY r = 0.17) levels. In contrast, it was negatively related to lysozyme activity (r = - 0.24) (see Fig. 3 and ESM6).

Structural model variance

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

Direct effects: Starting from the top of our constructs, individual diet composition was affected by individual age and environmental lagoon features encountered during the summer (Fig. 3; Table 6). The higher lagoon eutrophication, depth, area and DIN content, the more fish diet shifted from planktivorous, terrigenous, detrital, and EPA sources to carnivorous ones. The same relationship was found as fish age increased. Consequently, when fish shifted to a more carnivorous diet, structural lipid stocks decreased but growth increased (LV "fish structure"). Such diets had a positive effect on fish energy stores (reserve variable), reflected by increased TAG and TAG/PL ratio. Age showed opposite trends on fish reserve and fish structure: increasing age was associated with increasing structural lipids (although limited growth) but decreasing energy stores. We found no substantial effect of the LV "lagoon feature" on fish reserves or fish structure. Notably, fish structure was strongly affected by fish reserve with higher reserves leading to higher growth.

Fish age was also negatively associated with muscle pollutant concentrations, whereas fish reserve, structure and diet had non-significant effects on pollutant concentrations. Most

importantly, fish pollutant concentrations had the highest direct positive (+0.38) impact on fish stress. Increasing lagoon eutrophication, depth, area, DIN and decreasing salinity (LV "lagoon feature") were associated with increased fish stress (+0.25), whereas stress largely decreased with increasing fish age (-0.41). Finally, the direct effects of individual diet and energy stores (LV "Fish reserve") on individual stress was non-significant (Fig. 3, Table 6).

Indirect effects: The PLS-PM framework further allowed assessing indirect effects by multiplying coefficients along the path. Focusing on total effects affecting fish stress, positive direct effects of lagoon features (+0.25) on individual stress was counteract by negative indirect effects through muscle pollutant loads (total effect of lagoon features on pollutant load * total effect of pollutant on stress = -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 and ESM7). As a result, the overall effect of lagoon features on fish stress was low (+0.11). In contrast, direct and indirect age effects on fish stress were cumulative leading to the highest negative coefficient (-0.61): increasing age was associated with decreasing stress loads through direct and indirect effects. The indirect effects of fish diet, reserve and structure on fish stress were marginal (<0.06). A summary of direct and indirect effects is given Figure 4 (for a full description of the other LVs relationships see ESM7).

DISCUSSION

Using a multifactorial PLS-PM analysis, we identified key direct and indirect contributions of extrinsic (lagoon features, fish diet, fish pollutant loads) and intrinsic (fish reserves, fish structure components, fish age) factors affecting fish stress in coastal lagoons. Understanding

how individuals deal with multiple ecological pressures using stress as a proxy is a first step to better understand the constraints that apply to population dynamics (Brosset et al., 2021; McKenzie et al., 2016; Saraux et al., 2019). The approach allowed 33% of the total variance measured in ten Mediterranean lagoon systems to be explained. More importantly, 54% of the variance of the LV "fish stress" was explained by the dependent LVs "lagoon features", "fish age", "fish diet", "fish reserve", "fish structure", and "fish pollutant" included in the model.

Synergetic effects and consequences for individual body composition and growth

Lagoon features had an overall (direct + indirect) positive effect on fish reserve (+0.22) and fish structure (+0.28). Increasing eutrophication was positively linked to fish growth rates and the quantity of endogenous reserves individuals accumulated during the summer. This concurs with previous findings that seabream growth and body condition between and within lagoons generally increase with increasing organic matter inputs (Brehmer et al., 2013; Escalas et al., 2015; Isnard et al., 2015). These two effects were largely captured indirectly in our model (direct effects non-significant; coefficients < 0.05; Table 6) by dietary shifts from planktivorous, terrigenous, detritic, and EPA-based diets to carnivorous diets when eutrophication increases. Further, lagoon features and fish age appear to act synergistically on fish diet: as individuals age and grow, they progressively switch to bigger prey, developing a more carnivorous diet (Rosecchi, 1987).

Extrinsic and intrinsic factors affecting fish metal contaminant loads

Lagoon features had an important negative effect on fish pollutant loads. When the LV "lagoon features" increased, eutrophication (inorganic matter content and primary production) increased, while salinity decreased. This is consistent with the fact that both the toxicity of metal pollutants to fish and their bioavailability in water are known to vary with water

physicochemical properties, including pH, alkalinity, suspended solids, organic compound content, and hardness (Di Giulio and Meyer, 2008). Increasing organic matter concentration reduces metal accumulation to aquatic organisms by reducing the amount of free cationic metal by chelation (Richards et al., 2001; Schwartz et al., 2004). In rainbow trout, increased dissolved organic matter concentration has been associated to increased survival when faced with lethal Pb and Cu metal contaminations allowing reduced binding to gills (Richards et al., 2001; Schwartz et al., 2004). In contrast, while increasing salinity has been shown to reduce metal contaminant accessibility in bivalves and some teleost fish (Somero et al. 1977; Lee et al. 1998; Ifremer 2009), its biodisponibility in the water and sediments tend to increase (Zhao et al., 2013). Our results suggest that for seabream it could be due either to differences in gill osmoregulation processes in seabream compared to other species tested, or non-exclusively, to confounding effects of decreasing salinity and increasing organic matter concentration affecting pollutant loads. Indeed, the strong negative effect (-0.65) of the LV "lagoon feature" on fish pollutant load could be due to a stronger effect of increasing organic matter than decreasing salinity (higher loading and weight of Eutrophication of Lagoon features compared to Salinity; ESM6).

We also found that Gilthead seabream displayed a general pattern of metal contaminant bio-diminution with age. The PLS-PM showed that pollutant load decreased markedly with age (direct effect of fish age on pollutant load; -0.55, Fig. 3, Table 6). When focusing on individual metal concentrations and their respective relationships with the total length of individual (see ESM8), we found that aluminium (Al), boron (B), copper (Cu), nickel (Ni), rubidium (Rb), selenium (Se) and zinc (Zn) concentrations were all negatively related to total length. This suggests that dilution of those metals occurred with age-related changes in diet shift and size (Lee et al., 1998) and is consistent with similar relationships that have been described in trophic webs (cephalopods, coelenterates, crustaceans, echinoderm, fish, and gastropods) for cadmium

(Cd) and lead (Pb) in freshwater and marine ecosystems (Asante et al., 2008). In contrast, arsenic (As) and mercury (Hg) were the only two elements to bioaccumulate and/or biomagnify in seabream (reflected by positive correlations of their concentration with size). This seems to be common in marine organisms and food webs (Chouvelon et al., 2014; Cresson et al., 2015; Harmelin-Vivien et al., 2012). Cd, chromium (Cr) and Pb showed no relationship with total length (ESM8). This absence of relationships indicates that these metals were neither biomagnified nor biodiminished during ontogenetic development or through the food web.

Integrating multiple factors in our understanding of fish stress and health

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

et al., 2012; Scharf et al., 2000). Larger sizes may also be associated with reduced risks to predation (Sogard, 1997). Such long-term benefits may outweigh the short-term stress costs of investing in structural tissues and growth. We found that fish age (age in years and size) displayed the strongest direct effect on stress (-0.41). Older individual are thought to have greater experience since they have logically faced and survived several challenges as they age. Moreover, fish that survive longer may be of better intrinsic quality (biased selection), leading to individuals displaying lower maintenance costs in the sampled population (Birnie-Gauvin et al., 2017; Wilson and Nussey, 2010). These two non-exclusive hypotheses may explain the lower stress of older (max. 3 years old) individuals.

Interestingly, the effect of several lagoon features on stress was independent of the other studied LVs, *i.e.* not captured as indirect effects but as direct effect (+0.25). In other words, increasing eutrophication, lagoon morphology (depth and surface), inorganic nitrogen, and decreasing salinity directly increased individual stress. This may be due to direct effects on individual physiology, such as individual osmoregulation processes, that can be energetically stressful when salinity decreases and eutrophication increases (Bodinier et al., 2010; Cuesta et al., 2005). An alternative may be that these effects are captured indirectly, through other unmeasured lagoon ecosystem features known to increase stress and decrease immune functions, for instance predation risk (Werner et al., 1983) or competition (Goldan et al., 2008; Salati et al., 2016), which merit further investigation.

Most importantly, our analysis shows that gilthead seabream stress in coastal lagoon ecosystems was mostly affected by muscle inorganic pollutant content. Over the past decade, similar causal relationships have been shown by studies on contaminant loads under controlled laboratory conditions. Exposure to heavy metals is usually associated with increased cortisol and oxidative stress levels (Sevcikova et al., 2011) and lower immune function (Cerezuela et al., 2016; Guardiola et al., 2016, 2015). In small amounts, metal elements such as As, Cu, Cr,

Ni, and Zn are essential for immune and oxidative defence functions, whereas they impair these same functions in excess (Sevcikova et al., 2011). For instance, Zn or Cu deficiencies lead to deficiencies in immune and antioxidant systems and to the development of life threatening infections (Rink and Kirchner, 2000). In contrast, at high doses, those same elements have inhibiting effects on the immune system (Rink and Kirchner, 2000) or increase oxidative stress (Noh and Koh, 2000; Valko et al., 2005). Because of their deleterious impacts at low concentration on organism physiology, As, Cd, Hg and Pb are the most commonly (often individually) studied metal contaminants. Here, we considered a wider array of pollutants, considering cocktail effects in interaction with other environmental features rather than single contaminant effects. It is remarkable that individual contaminant loadings on the LV "pollutant load" were relatively low for these widely studied metal pollutants (As +0.14, Cd +0.02, Hg +0.20 and Pb +0.10). Rather, it appeared that interactions between B (+0.50), Cu (+0.57), Rb (+0.35), Zn (+0.46), Al (+0.40) and a decrease in Se (-0.56) were critical in explaining individual stress in seabream. It is important to understand that the physiological action of these two different groups of pollutants in causing oxidative stress are fundamentally different (Valko et al., 2005). Metals without redox potential, such as As, Cd, and Hg, cause oxidative stress by impairing antioxidant defences, especially those involving thiol-containing antioxidants and enzymes (Stohs and Bagchi, 1995). In contrast, redox active metals such as Fe, Cu and Al participate in the formation of reactive oxygen species (*e.g.* Fe and Cu catalyse the formation of reactive hydroxyl radicals (•OH) by Fenton reaction) (Gaetke and Chow, 2003; Liaquat et al., 2019). Elements such as dietary Se have been shown to protect against lipid peroxidation and Cu (Kadiiska and Mason, 2002) and Cd (Ulusu et al., 2003) toxicity. The above highlights the complexity of interactions between pollutants, and the importance of integrated approaches to evaluate cocktail effects. Over the last decade, pollution monitoring programs for coastal ecosystems were developed for the Mediterranean Sea to provide scientific knowledge to assess

ecosystem health and sustainability (Bonito et al., 2016; Naccari et al., 2015; Tomasello et al., 2012). However, mainly due to European legislation, most studies on fish species have focused on As, Cd, Hg and Pb contamination or have compared contaminant loads for different species at different locations (Cresson et al., 2016; Mille et al., 2018; see Chouvelon et al., 2017 for a multi-population approach for tuna), although few studies have focused on the consequences on individual health in nature. Yet, as monitoring of organic contaminants such as POPs (HAPs, PFOS, PFAS, DDT, Lindane…) become more common in marine environments, it urges further research that would certainly bring new insights on the impact of changing lagoon environment on fish health (Munaron et al., 2023).

Conclusion

Using the Gilthead seabream and Mediterranean lagoons as a model, our study emphasized the importance of integrated approaches to identify direct and indirect contributions of multiple environmental factors affecting individual stress in coastal ecosystems. Abiotic and biotic features of the environment have complex direct and indirect, synergetic or antagonistic, interactions with individual intrinsic features. A proper evaluation of these effects on health requires multi-factorial frameworks. The challenge ahead lies in extending such approaches from the individual to population scale.

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AUTHORS CONTRIBUTION

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

COMPLIANCE WITH ETHICAL STANDARDS

All experiments were approved by the national ethic comitee (APAFIS#8945-20l 7021612469374 v4 & APAFIS#10759-2017072512353814 v3) and realized in compliance with French and European Law.

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FIGURE CAPTIONS

Figure 1. Path diagram of the structural model.

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

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

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

TABLE CAPTIONS

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

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

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

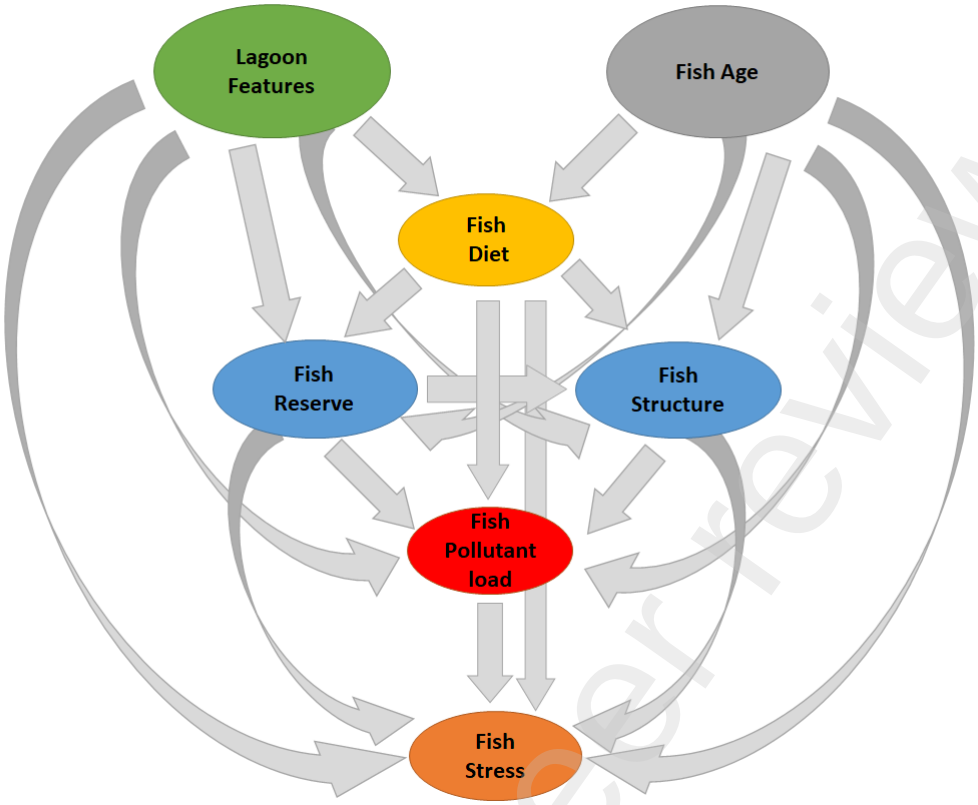
Table 4. Summary of the different parameters measured in fish, the tissue considered, their unit, their role as biomarkers and the latent variable they refer to.

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

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

FIGURES

Figure 1.



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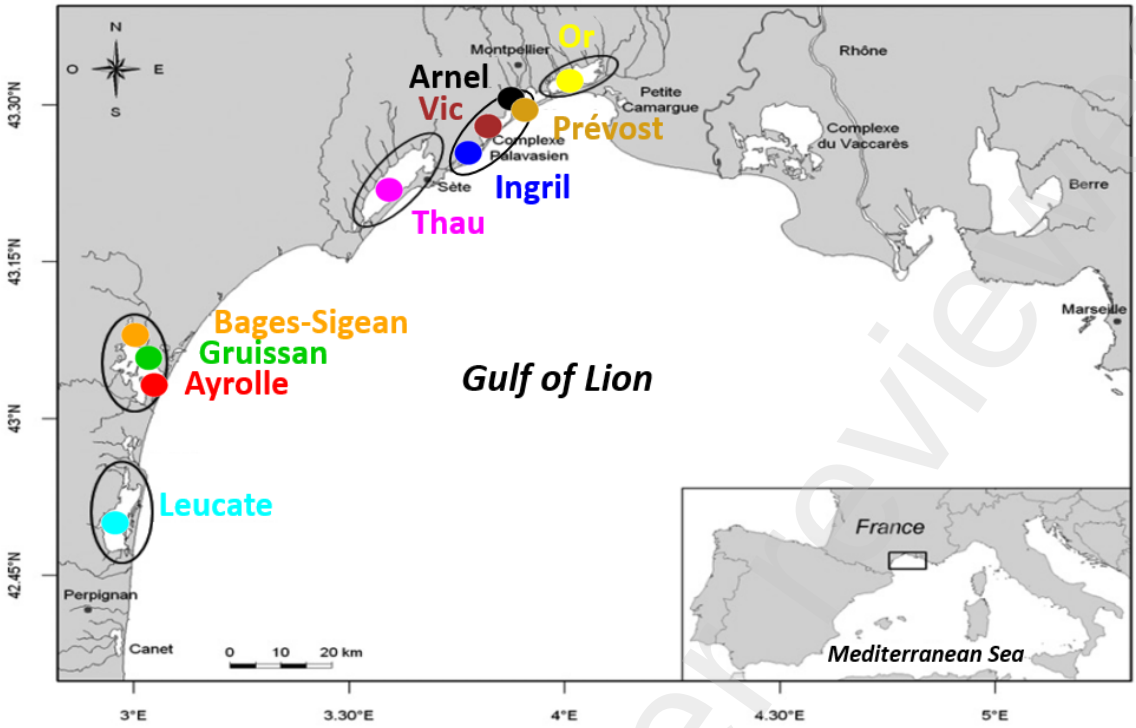
1211 **Figure 2.**



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1223 **Figure 3.**

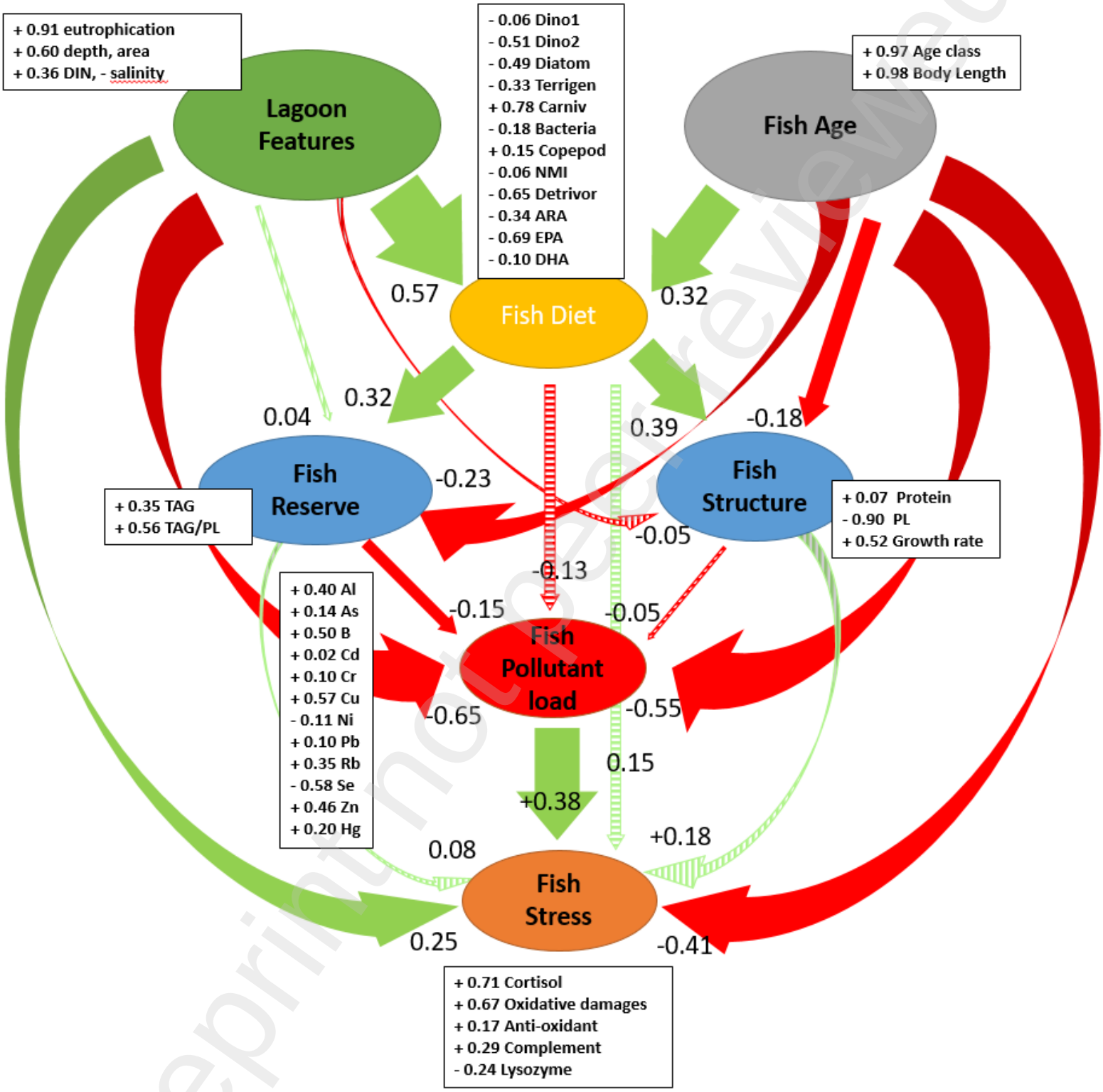

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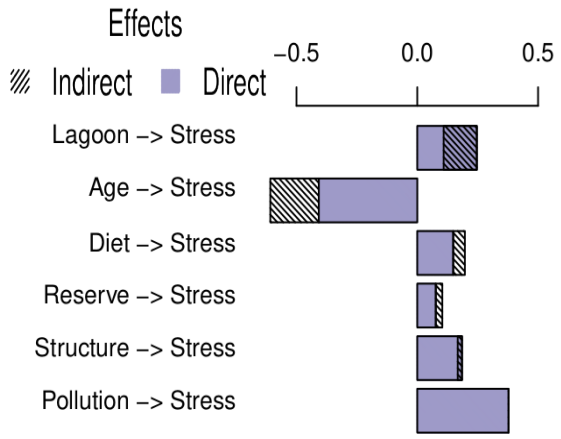
Figure 4.



Direct	Indirect	Total
0.25	-0.14	0.11
-0.41	-0.20	-0.61
0.15	0.05	0.20
0.08	0.03	0.10
0.18	-0.02	0.17
0.38	0.00	0.38

TABLES

Table 1.

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

1237 **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: 16:1w7/16:0	Dinoflagellates	(St John and Lund, 1996)
Dino2: 20:5w3/22:6w3	Diatoms vs dinoflagellates	(Budge and Parrish, 1998)
Diatom : 16:1w7/20:5w3	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: 20:1w9 + 22:1w11	Copepods	(Budge et al., 2002; Kattner et al., 2012)
Terrigenous: 18.2w6 + 18.3w3	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: 18:1w9/18:1w7	Carnivorous	(Auel et al., 2002; El-Sabaawi et al., 2009; Graeve et al., 1997)
Essential FA	**Role**	**Reference**
Docosahexaenoic acid (DHA): 22:6ω3	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): 20:4ω6	Essential FFA: precursor of eicosanoid hormone	
Eicosapentaenoic acid (EPA): 20:5ω3	Essential FFA: precursor of eicosanoid hormone	

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1242 **Table 3.**

						Lagoon	Diet		Reserve		Structure		Pollutant		Stress		Age	
Model	***Model specificity***	*Lvs*	*Mvs*	*Direct Paths*	*GOF*	Block	Block	R^2	Block	R^2	Block	R^2	Block	R^2	Block	R^2	Block	R^2
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	
2	**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
3	**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	
4	**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	
5	**Basic + efffect 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	
6	**Basic + efffect 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	
7	**Basic + efffect 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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1244 **Table 4.**

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Parameters	**Tissue**	**Unit**	**Role**	**Latent Variable**
Cortisol	Scales	$\mu g.g^{-1}$	Systemic stress hormone	Fish Stress
Oxidative damages	Plasma	Mg H_2O_2 equivalent.dL^{-1}	Oxidative damages accumulated	Fish Stress
Antioxidant	Plasma	µmol 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 pollutants	Muscle	$\mu g.g^{-1}$ dry muscle	Contamination load by each inorganic pollutants	Fish Pollutant
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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LATENT VARIABLE	Type	Manifest variables	Block R^2	Communality
Lagoon features	Exogenous	↑ Eutrophication, depth & area and DIN ↓Salinity		**0.439**
Fish Age	Exogenous	↑ Age ↑ Fish total length		**0.943**
Fish Diet	Endogenous	↑ Carnivorous, Copepods ↓ Dinoflagellates, Diatoms 1 & 2, Terrigenous, Bacteria NMI, Detrital, ARA, EPA, DHA	**0.283**	**0.193**
Fish Reserve	Endogenous	↑ TAG and TAG/PL	**0.160**	**0.212**
Fish Structure	Endogenous	↑ Growth rate ↓ PL	**0.550**	**0.359**
Fish Pollutant load	Endogenous	↑ Al, As, B, Cr, Cu, Rb, Zn, Hg ↓ Se, Ni r≤**0.10** Cd, Cr, Pb	**0.626**	**0.124**
Fish Stress	Endogenous	↑Cortisol, oxidative stress damages, anti-oxidant, complement activity ↓Lysozyme activity	**0.538**	**0.225**
Goodness of fit	**0.3262**			

Table 6.

	Estimate	Std. error	t value	P value
$ Fish Diet				
Intercept	0.000	0.088	0.000	1
Lagoon features	0.571	0.096	5.96	4.48e-8*
Fish Age	0.321	0.096	3.65	1.15e-03*

$ Fish Reserve				
Intercept	0.000	0.096	0.000	1
Lagoon features	0.037	0.123	0.302	0.763
Fish Age	-0.231	0.110	-2.10	0.039*
Fish Diet	0.318	0.113	2.82	0.006*
$ Fish Structure				
Intercept	0.000	0.070	0.000	1
Lagoon features	-0.048	0.090	-0.329	0.596
Fish Age	-0.183	0.083	-4.370	0.030 *
Fish Diet	0.391	0.087	3.680	1.84e-05 *
Reserve	0.499	0.077	6.50	4.28e-09
$ Fish Pollutant load				
Intercept	0.000	0.064	0.000	1
Lagoon features	0.650	0.083	-7.840	8.56e-12*
Fish Age	-0.555	0.078	-7.090	2.94e-10*
Fish Diet	-0.132	0.088	-1.510	0.135
Fish Reserve	-0.149	0.085	-1.750	0.084
Fish Structure	-0.047	0.096	-0.485	0.629
$ Fish Stress				
Intercept	0.000	0.072	0.000	1
Lagoon features	0.246	0.120	2.050	0.043*
Fish Age	-0.408	0.109	-3.730	3.3e-4*
Fish Diet	0.149	0.099	1.500	0.138
Fish Reserve	0.076	0.097	0.790	0.432
Fish Structure	0.185	0.107	1.720	0.089*
Fish Pollutant load	0.378	0.118	3.210	0.002*

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