Spatial and ontogenetic modulation of fatty acid composition in juvenile European sea bass (Dicentrarchus labrax) from two French estuaries

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Abstract

This study compared the fatty acid (FA) composition in the liver, muscle, and brain tissues of wild European sea bass juvenile from two French estuaries (Loire and Seine), focusing on the variability across ontogenetic stages (first, second and third year, i.e. G1, G2, G3, respectively). We highlighted tissue-specific patterns, with the brain exhibiting a distinct FA composition from the two other tissues. Ontogenetic stage and estuary influenced the general FA profile, and particularly essential FA (EFA) like DHA, eicosapentaenoic acid (EPA), and arachidonic acid (ARA) in all tissues. The data also revealed the ability of wild sea bass to modulate, at molecular level, FA biosynthetic pathways, and suggests a potential dietary DHA deficiency in the natural environment, especially for Seine G1 juveniles. The differential distribution of FA within tissues might reflect shifts in diet, metabolic demands, or adaptations to environmental conditions. The study provides insights about FA dynamics in euryhaline fish during juvenile life stages, enhancing our understanding of their metabolic and trophic interactions, and underscoring the need to further investigate potential effects of FA depletion in a changing trophic environment.

Key words: Lipid composition, fish, euryhaline DHA, EPA, ARA

1. Introduction

Long-chain polyunsaturated fatty acids (≥ 20 carbon atoms, LC PUFA), particularly eicosapentaenoic acid (EPA; 20:5-n-3), docosahexaenoic acid (DHA; 22:6n-3), and arachidonic acid (ARA; 20:4n-6), are necessary for numerous biological functions in organisms, and are named essential fatty acids (EFA) (Cottin et al., 2011). They are the major components of cell membrane phospholipids (polar lipids, PL), influencing membrane fluidity, permeability, and functionality (van Meer et al., 2008). They are also found in lesser proportions in triglycerides (neutral lipids, NL), which are a source and reserve of energy. EPA and ARA serve as substrates for eicosanoid synthesis, a group of potent signaling molecules that mediate numerous physiological processes, including inflammation, immune response and reproduction (Calder, 2017; Gómez-Abellán and Sepulcre, 2016). These fatty acids (FA)

are distributed in a highly compartmentalized manner across different organs, reflecting their varied roles in the organisms. High concentrations of DHA and ARA are usually found in neural tissue phospholipids, indicative of their crucial roles in brain development and function (Mejri et al., 2021) while the muscle, essential for locomotion, usually exhibits high levels of EPA (Tocher, 2003). Studying NL and PL fatty acid composition can give insights about how storage and structural lipids are regulated in tissues, and can be linked to physiological performances within an individual (Twining et al., 2020).

LC PUFA, and especially LC n-3 PUFA are naturally synthesized by aquatic microalgae at the basis of the food chain (Maltsev and Maltseva, 2021). Fish, as consumers, rely on the dietary supply of these nutrients due to their limited LC n-3 PUFA biosynthetic capacity (REF). The enzymes involved in the biosynthesis process are the fatty acyl desaturases (*fads*) and elongases of very long-chain fatty acids (*elovl)* (Monroig et al., 2010) (Fig. S1). While the liver is the primary site for lipid biosynthesis both liver and brain have shown LC-PUFA biosynthesis abilities (Galindo et al., 2021; Monroig et al., 2018). This LC-PUFA biosynthesis has been shown to be modulated by environmental factors such as temperature (Tocher et al., 2004), salinity (Zheng et al., 2005) and diet composition (Turchini et al., 2011). Fish may also upregulate the expression of genes involved in the synthesis of LC PUFA to partially compensate for dietary deficiencies (Glencross, 2009; Vagner et al., 2007b). Yet, this upregulation at molecular level may not be sufficient to compensate for dietary deficiency in the tissue FA composition (Vagner et al., 2009, 2007a)

Recent environmental changes of temperature, ocean pH, and oxygen concentration, pose a significant threat to aquatic ecosystems (Gattuso et al., 2015; Pörtner et al., 2022). These environmental alterations can lead to shifts in microalgae species assemblages and affect their physiology, ultimately resulting in a decrease in LC PUFA production at the base of the marine food web (Galloway and Winder, 2015; Hixson and Arts, 2016; Poloczanska et al., 2013). This would lead to a lower LC PUFA availability for consumers, such as fish, and therefore limiting their ability to adjust the LC-PUFA composition and thus functionality of their membrane (Brett et al., 2009). A reduced LC PUFA content in fish cell membranes has been found to have profound cascading effects on fish physiology, including reduced growth (Vagner et al., 2014), altered energy metabolism, reduced immune function, and impaired reproductive success (Bell and Koppe, 2010; Schmitz and Ecker, 2008; Vagner et al., 2019, 2015, 2014). The consequences of these physiological changes may translate to population level, affecting ecosystem structure and functioning (Poloczanska et al., 2013).

The European sea bass (*Dicentrarchus labrax*) is a key species in the coastal and estuarine ecosystems of the Atlantic Ocean and Mediterranean Sea. This species has a complex life cycle, with juveniles utilizing estuaries as nurseries to grow and mature, and adults migrating to offshore waters for feeding and reproduction (Pawson et al., 2000). The distribution of sea bass within estuaries can be highly variable and influenced by numerous factors such as temperature, salinity or food availability (Blaber and Blaber, 1980; Pawson and Pickett, 1996). It is known to be an opportunistic predator that feeds on the most abundant prey available (Pérez-Ruzafa and Marcos, 2014). As fish grows, their dietary preferences shift, and they target larger prey potentially having different FA compositions, influencing the FA profile of the fish.

Despite intensive research on effects of dietary FA in sea bass (Geay et al., 2010; Torrecillas et al., 2017), little is known about the LC-PUFA metabolism of wild individuals, most of the research being focused on the comparison of fatty acid composition between wild and farmed sea bass (Bhouri et al., 2010; Fuentes et al., 2010; Orban et al., 2003; Tarricone et al., 2022).

The Seine and Loire estuaries, located along the French Atlantic coast, are essential estuaries for the European sea bass, providing suitable environmental conditions for their growth, survival, and larvae recruitment (Beck et al., 2001; Le Pape et al., 2003). These estuaries are characterized by highly productive ecosystems, driven by nutrient inputs from their respective rivers and the coastal waters, which support diverse assemblages of phytoplankton, zooplankton, and organisms of higher trophic levels (Ménesguen et al., 2018; Vasconcelos et al., 2015). They can exhibit broad differences in temperature, salinity or nutrient availability that modulate the communities within the estuary (Selleslagh et al., 2009). The Seine and Loire estuaries are also exposed to various anthropogenic pressures, such as urbanization, agriculture, and industrial activities, leading to the degradation of water quality, loss of essential habitat, disruption of food web dynamics and overall affecting the survival of juvenile fish (Le Pape et al., 2007; Ménesguen et al., 2018; Teichert et al., 2016) Climate change is expected to exacerbate these pressures by altering temperature, precipitation, and sea level, potentially affecting the functioning of estuarine ecosystems and the life cycle of the European sea bass (Pörtner et al., 2022).

This study aimed to address critical knowledge gaps regarding the adaptive capacity of fish to changing environmental conditions, with a focus on the role of FA in this process. Specifically, we hypothesized that (1) different organs will exhibit distinct FA profiles related to their physiological roles; (2) the ontogenetic stage and estuary of origin will influence these FA profiles, reflecting age-related differences in metabolic needs and foraging patterns; and (3) a relationship exists between LC PUFA profiles in fish tissue and the expression of genes involved in lipid metabolism. To test these

hypotheses, our investigation focused on the LC PUFA profiles of the liver, muscle, and brain of juvenile wild sea bass from the Seine and Loire estuaries, together with the molecular modulation of LC n-3 PUFA biosynthesis pathways.

2. Material and Methods

Ethical statement

Authorization and ethical approval for fish sampling were provided by national (DPMA) and regional authorities (Normandie, Pays de la Loire); National & regional committees of professional fishermen (CNPMEM, CRPM Normandie; COREPMEM Pays de la Loire) in 2019 (Ref. Osiris PFEA400018DM0310001; ref. Ifremer: 18/2216441). All fish analyzed were dead by the time of tissue sampling.

2.1. Studied sites and sample collection

Juvenile European sea bass were sampled in the Loire estuary for 3 days in July 2019 and in the Seine estuary for 3 days in August 2019 during an annual NOURDEM survey funded by Ifremer (French Institute for Sea research and Exploitation). Samplings were performed from upstream to downstream of the estuary (for zones of capture, see Fig. S2). A bottom otter trawl (7m wide, 2.40m high), specifically designed to capture demersal fish juveniles, was used to catch the fish (Le Goff et al., 2022). Following each trawl, the catch was sorted to retain only sea bass individuals aged from 1 to 3 years (G1, one-year old, 12-20 cm; G2, 2-years old, 20-27 cm and G3, three-years old, 27-34 cm), based on their length according to the length distribution referential implemented during the NOURDEM survey. During the trawling, a probe measured the temperature and salinity (Table S1). The collected sea bass were then euthanized using MS-222 (400 $mg.L^{-1}$). We measured fish total length (nearest 0.1 cm), weight (nearest gram) and sampled a few scales to confirm the age of the different fish. Brain and liver were entirely removed. About 200 mg of muscle were taken on the left side of the fish, dorsally from the lateral line and just behind the head. Gallbladder was removed from the liver. All samples were immediately flash frozen in liquid nitrogen until reaching the lab, where they were stored at -80°C pending further analysis. A total of 76 individuals were collected: 12 for Seine G1 (SG1), 18 for Loire G1 (LG1), 10 for Seine G2 (SG2), 12 for Loire G2 (LG2), 12 for Seine G3 (SG3) and 12 for Loire G3 (LG3).

2.2. Life history traits measurements

We calculated Le Cren body condition factor (CF) (Le Cren, 1951) which is defined as the ratio between the weight of the fish and a theoretical weight for its length obtained using observations of the population :

$$K_n = W/aL^b$$

Where Kn is the Le Cren body condition factor, W is the observed mass, L the observed length and a and b are constants estimated from the length-weight relationships. This relationship was established a single time by pooling the fish from both estuaries and all of the age classes.

2.3. FA profiles analysis

2.3.1. Sample preparation and lipid extraction

Prior to any manipulation, all of the glassware was heated to 450°C for 6h and the metal or Teflon material were rinsed using acetone to prevent contamination of the samples. Frozen tissues (n=75 liver, n=50 muscle and n=50 brain, for detail, see Table 1) were grounded in liquid nitrogen into a homogeneous powder and dived into a 6 mL mixture of chloroform/methanol (2:1, v/v) using from 50 to 200 mg of wet weight of powder. To optimize the lipid extraction, all of the extracts were sonicated for 10 min and agitated for 20 min before being stored at -20°C under nitrogen atmosphere prior to further analysis.

Table 1: Number of samples for lipid analysis for each tissue. One sample in Seine G1 was excluded from analysis after being considered an outlier.

Group	**Liver**	**Muscle**	**Brain**
Loire G1	18	10	10
Loire G2	11	8	8
Loire G3	12	8	8
Seine G1	12	8	7
Seine G2	10	8	8
Seine G3	12	8	8

2.3.2. Lipid separation

For all the samples, lipids were separated into a neutral (NL) and polar (PL) fraction following the method described by Le Grand et al. (2014). An aliquot (from 750 to 3000 µL, depending on the sample biomass) of the total lipid extract was evaporated to dryness, re-suspended three times using 500 µL of a mixture of chloroform/methanol (98:2, v/v) and deposited at the top of a silica gel (40 mm x 4 mm, silica gel 60A 63-200 µm rehydrated using 6% H_2O (70-230 mesh)). NL were eluted using10 mL of a mixture of chloroform/methanol (98:2, v/v) and PL were then eluted using20 mL of

methanol. After the elution, 2.3 µg of an internal standard (tricosanoic acid, C23:0) was added to each fraction that was then evaporated to dryness using a Genevac centrifugal evaporator. 1600 µL of H_2SO_4/MeOH (3.4%) were added and the samples were incubated for 10 mn at 100°C to form FA methyl esters (FAME). FAMEs were extracted by adding 800 µL of hexane and 1500 µL of hexane-saturated distilled water and by shaking and centrifuging both fractions 1 min at 738g at room temperature. The aqueous phase was removed and the organic phase, containing the FAME was washed two more times using hexane-saturated distilled water.

2.3.3. FAME analysis

FAMEs were analyzed in a Varian CP8400 gas chromatograph (GC) coupled with flame ionization detector (FID) as described in Mathieu-Resuge et al., (2019). FAMEs were injected in splitless-mode in parallel on two different columns (DBWAX 30m x 0.25 mm ID x 0.2 µm and DB5 30 m x 0.25 mm ID x 0.2 µm, Agilent). Identification of FAME was realized by comparison of their retention times based on those of commercial standards (Supelco, 37 Component FAME mix, PUFA N°1 and N°3, and Bacterial Acid Methyl Ester Mix, Sigma). Internal standard allowed to calculate FA content ($\mu g.mg^{-1}$ WW). Fatty acid proportion was defined as the mass percentage of each fatty acid to the total fatty acid content. For the brain, we focused on PL FA in the brain as NL fraction accounts for less than 20% of the total FA (data not shown) and are less scientifically relevant.

2.4. Gene expression analysis

Total RNA were extracted from the liver powder (n=75; see Table 1 for details) using Extract-all reagent (Eurobio; Courtaboeuf, Essonne, France) coupled with purification steps on a Nucleospin RNA column as described by Mazurais et al., (2020). The extraction protocol included one-step of DNase treatment (Macherey-Nagel, Düren, Germany). Concentrations and purity of extracted RNA were measured using a ND-1000 NanoDrop spectrophotometer (ThermoScientific Inc., Waltham, MA, USA). An Agilent Bionanalyzer 2100 (Agilent Technologies Inc, Santa Clara, CA, USA) was used to evaluate the RNA integrity (RIN) and 72 samples had a RIN higher than nine.

Two positive and one negative reverse transcription (RT) reactions for cDNA synthesis were performed using iScript cDNA Synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA, USA) as described in Mazurais et al. (2020). The relative expression levels of following transcripts were investigated (Table S2): Fatty acid desaturase 2 (*fads2*), Lipoprotein lipase (*lpl*), Group XIIB secretory phospholipase A2 (*plag12b*), Stearoyl-CoA desaturase 1b (*scd1b*) and Succinate dehydrogenase cytochrome b560 subunit (*sdhc*). These genes were chosen because they are involved in the lipid or LC-PUFA metabolism (Rimoldi et al., 2016). The primers used, as well as the GENBANK sequence

numbers are presented for each gene in Table S2. The relative quantity of these transcripts of interest and those of three housekeeping genes (elongation factor 1-alpha, *ef1*; Beta Actin, *actin* and Ribosomal protein L13a, *l13a*) was determined by qPCR using a CFX96 Touch Real-Time PCR Detection system (Bio-Rad Laboratories Inc.). The relative quantities of transcripts were normalized using the ΔΔCt method (Livak and Schmittgen, 2001).

2.5. Statistical analysis

All analyses were conducted on RStudio (V4.2.1). The multivariate approach used for the general fatty acid profile was realized using a PERMANOVA followed by pairwise tests (package *vegan*, *pairwise adonis* function) to test for significant differences in the general FA profile between the different groups within each tissue. The FA for the multivariate analysis were selected based on a similarity percentage analysis (SIMPER, Clarke, 1993). to identify the major FA contributing to differences between groups. Differences between estuaries and between ontogenetic stages for weight, total length, condition factor, specific FA or gene expression were tested using a two-way ANOVA. When the Estuary*Stage interaction was significant ($p<0.05$), a new variable "Group", combining Stage and Estuary, was created (*e.g* Seine G1). A one-way ANOVA was then performed followed by a Tukey post hoc test to account for differences between groups. When the Estuary*Stage interaction was not significant ($p>0.05$), the differences between groups were tested following the model: X~Estuary+Stage where X is the tested variable (*e.g* DHA). A multiple-comparison test (package *multcomp*, *glht* function) was used to account for differences between groups. When the one-way ANOVA conditions were not met, a Kruskal-Wallis test was used instead.

3. Results

3.1. Weight, length and condition factor

For both estuaries, an increase in mean fish weight and length was observed from G1 to G3 (Table 2). However, the Loire fish consistently had a greater weight and length than Seine fish at each stage. These differences were statistically significant for both stage ($p<0.001$) and estuary ($p<0.001$). The CF, however, remained constant across developmental stages for both estuaries and no significant differences were observed in relation to either the stage or estuary.

Table 2: Weight (g), Total Length (TL, cm) and LeCren condition Factor (K_n) of the juvenile European sea bass from the different ontogenetic (G1: one year old; G2: two years old; G3: three years old) and location (Seine and Loire estuaries) groups. Values are expressed as mean ± SEM. Potential differences among groups were assessed by 2-way ANOVA and Tukey's post hoc test. Main effects are given in the right columns – Stage: effect of the life stage; estuary: effect of the sampling site; Stage x estuary: interaction of the two. Significance was accepted at $p < 0.05$. Values within each line not sharing common letters are significantly different: *** $p < 0.001$, ** $p < 0.01$, - NS. Loire : G1 (n=17), G2 (n=12), G3 (n=12). Seine : G1 (n=11), G2 (n=10), G3 (n=12). One fish was not measured.

	LOIRE			SEINE			STATISTICS		
	G1	G2	G3	G1	G2	G3	Stage	Estuary	Interaction
Weight	44.8 ± 2.9^{b}	162.7 ± 6.2^{d}	307.4 ± 17.9^{f}	38.6 ± 2.8^{a}	137.5 ± 6.0^{c}	270.1 ± 15.2^{e}	***	***	-
TL	16.5 ± 0.3^{b}	25.3 ± 0.3^{d}	31.1 ± 0.6^{f}	15.3 ± 0.4^{a}	23.9 ± 0.4^{c}	29.8 ± 0.5^{e}	***	**	-
K_n	0.96 ± 0.04	1.01 ± 0.03	1.01 ± 0.01	1.08 ± 0.02	1.01 ± 0.02	1.01 ± 0.02	-	-	-

3.2. General FA profile

The main FA family found in PL of the three tissues was PUFA. Muscle and liver displayed higher PUFA proportions compared to the brain (54, 53 and 33% respectively; detailed fatty acid compositions of both NL and PL fractions of the three organs are presented in Table S2). DHA (22:6n-3) was the major PUFA in PL in the three tissues followed by EPA (20:5n-3) (Fig. 1A). The brain displayed the highest average monounsaturated fatty acid (MUFA) concentration (30 ± 0.4%) in PL among tissues, which was more than twice that of the liver and muscle (14 ± 0.2 %). The oleic acid (18:1n-9) was the major MUFA in the three tissues and its highest concentration was found in the brain (18 ± 0.2%) (Fig. 1A). Interestingly, the 24:0 and 24:1n-9 were significantly higher in the brain compared to muscle and liver (2% vs 0.1% (for both muscle and liver) for 24:0 and 4% vs 1.2% and 0.7% for 24:1n-9, respectively). The mean concentration of saturated fatty acid (SFA) in PL was similar among the three tissues (about 29%) where 16:0 and 18:0 were the predominant FA (Fig. 1A).

In NL, only liver and muscle were analyzed and the proportions of the three FA families (SFA, PUFA, PUFA) were relatively similar between both tissues. A balanced distribution among FA families was observed in muscle, with relatively close concentrations: SFA (30%), MUFA (32%), and PUFA (33%) with 16:0, 18:1n-9, EPA and DHA as major FAs in both tissues. Liver had the highest concentrations of MUFA (40%; Fig. S2 B) with 18:1n-9 being the major FA (Fig. 1B).

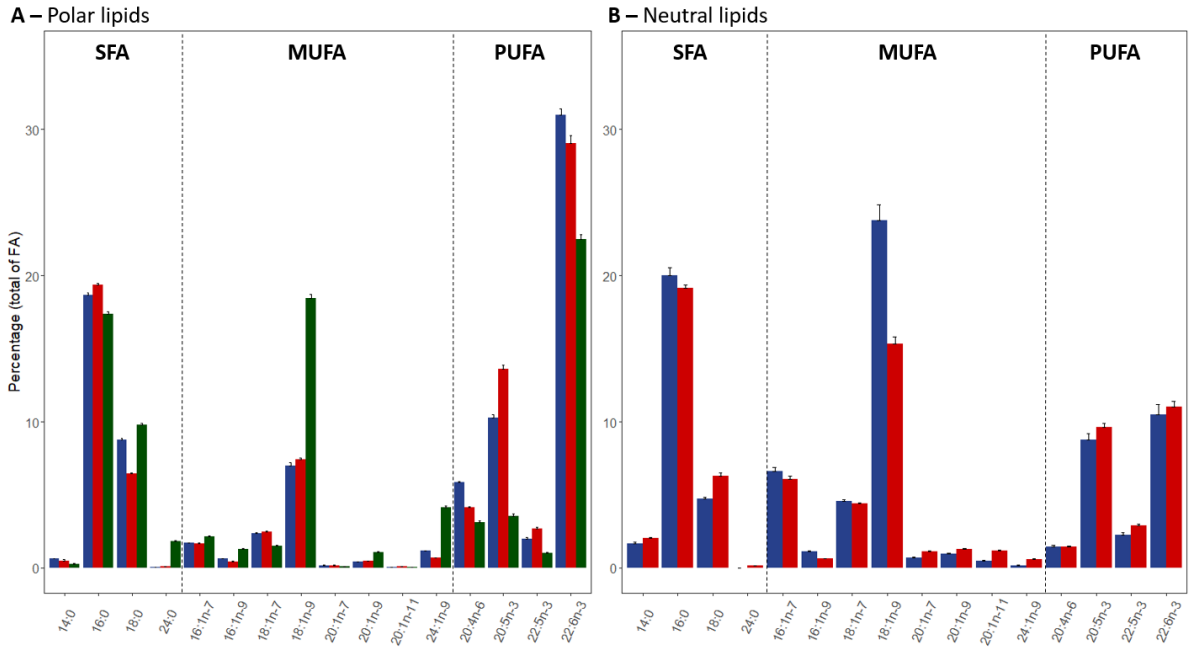

Figure 1: Proportions of fatty acids (mass percentage of total FA) in polar lipids (A) and neutral lipids (B) in the liver (blue, n=75), muscle (red, n=50) and brain (green, n=49) of juvenile European sea bass from all confounded Seine and Loire locations and ontogenetic stages. Only FA that are >1% for at least one tissue are presented. Data are presented as mean ± SEM. Neutral lipids have not been measured in the brain (cf material and method section for details). SFA = Saturated Fatty Acid, MUFA = Monounsaturated Fatty Acid, PUFA = Polyunsaturated Fatty Acid.

The inertia of FA composition in fish PL, according to their ontogenetic stage and sampling location is presented in the PCA in Fig. 2.

In the liver (Fig. 2A), significant differences in PL FA composition were found among the groups (PERMANOVA, Table S3, 4). In the Seine estuary, the G1 from Seine were different from the two other ontogenetic groups and appeared to be distinguished, among others by their EPA (20:5n-3) proportions, while G2 and G3 seemed to be distinguished by their DHA (22:6n-3) proportions (Fig. 2A). However, that trend was not observed in the Loire estuary.

In muscle (Fig. 2B), significant differences were found among the groups (PERMANOVA, Table S3, 4). The Seine G1 group was different from all the other groups except from Seine G2. The first dimension distinguished the stages, with G1 being characterized by EPA and G2 and G3 being characterized by DHA. The second dimension distinguished the estuaries, with Seine that tended to be characterized by 16:0 and 18:0, and Loire by 22:5n-6 and ARA (20:4n-6). Brain FA composition in PL (Fig. 2C) was impacted by an interactive effect between site and ontogenetic stages, with the Seine G2 group being different from Loire G1 and Loire G2 (PERMANOVA, Table S3, 4). The G1 seemed to be distinguished by EPA and DPA (22:5n-3) and the G3 by ARA and 16:1n-9.

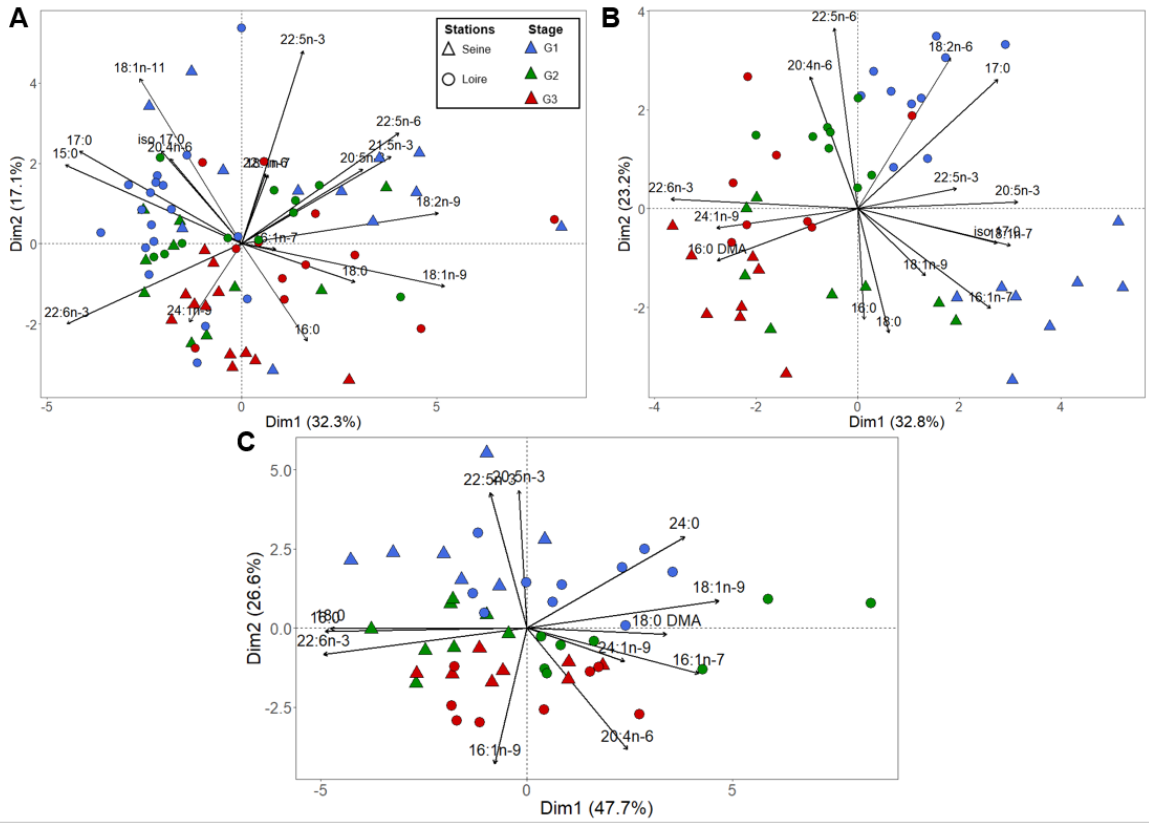

Figure 2: Principal Component Analysis (PCA) of polar lipid fatty acids in liver (A), muscle (B) and brain (C) of 1 (G1), 2 (G2) or 3 (G3) years old juvenile European sea bass from Seine and Loire. Only FAs that account for >80% of the contribution of dissimilarity between groups are shown (SIMPER test) Liver : Loire G1 (n=18), Loire G2 (n=11), Loire G3 (n=12), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12). Muscle : Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=8), Seine G2 (n=8), Seine G3 (n=8). Brain: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=7), Seine G2 (n=8), Seine G3 (n=8).

3.3. FA proportions and ratios between ontogenetic stage and estuaries

3.3.1. DHA proportions

In the liver (Fig. 3A), DHA proportions followed different dynamics in the two estuaries. It decreased with the ontogenetic stage in Loire, while it tended to increase in Seine. In muscle (Fig. 3 B), DHA significantly increased with the ontogenetic stage in Seine, while it remained similar in all ontogenetic groups in Loire. Seine G1 displayed a lower DHA proportion than all other groups. In the brain (Fig. 3C), the DHA content did not differ among the ontogenetic stage in Seine. The G2 in Loire had a lower DHA content than the G3 in Loire and was lower than all ontogenetic stages of Seine.

3.3.2. EPA proportions

For the liver (Fig. 3A) in Loire, the EPA proportions followed an opposite pattern to that of DHA, with Loire G3 having higher proportions compared to Loire G1. It was also significantly higher than in Seine G2 and Seine G3. However, EPA proportions remained stable in groups of Seine. In muscle and

brain (Fig. 3B, C), EPA proportions were significantly impacted by ontogenetic stages similarly in both estuaries, with significantly higher EPA proportions in G1 than in G2 and G3.

3.3.3. DHA/EPA ratios

In the liver (Fig. 3A), the DHA/EPA ratio followed the same trend as the DHA proportions (Fig. 4 A). In muscle (Fig. 3B), DHA/EPA ratio increased from G1 to G3 in both estuaries. In the brain (Fig. 3C), the DHA/EPA ratio was not different among the Seine groups, while in Loire it was lower for G1 and G2 compared to G3.

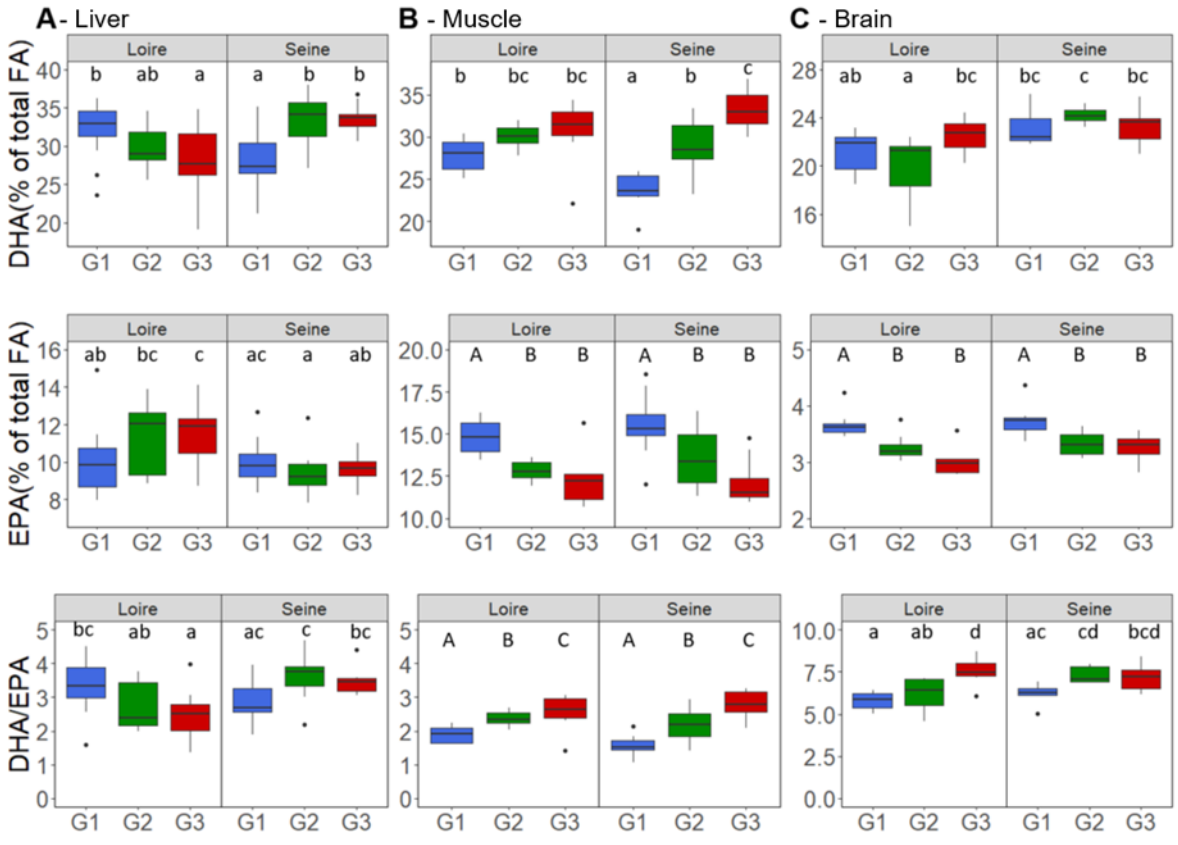

Figure 3: Proportions of DHA and EPA (percentage of total FA) and DHA/EPA ratio in the polar lipids of liver (A), muscle (B) and brain (C) of juvenile European sea bass from Seine and Loire. Different letters within a plot indicate significant differences. Letters in lowercase represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc) and uppercase letters represent a significant difference between either stage or estuary without significant interaction. Significance was accepted at $p < 0.05$. Liver: Loire G1 (n=18), Loire G2 (n=11), Loire G3 (n=12), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12). Muscle: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=8), Seine G2 (n=8), Seine G3 (n=8). Brain: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=7), Seine G2 (n=8), Seine G3 (n=8).

3.3.4. ARA proportions

In all tissues, ARA proportions were higher in Loire than in Seine. In the liver (Fig. 4A), ARA proportions were higher in G2 than G1 in both estuaries. In muscle (Fig. 4B), it did not differ between

ontogenetic stages in any estuary, while in the brain (Fig. 4C), it increased consistently with stages in both estuaries.

3.3.5. EPA/ARA ratios

In the liver (Fig. 4A), no statistical difference was found for the EPA/ARA ratio. In the muscle and brain (Fig. 4B, C), the EPA/ARA ratio was higher in the Seine than in Loire and G1 had a higher ratio than G2 and G3.

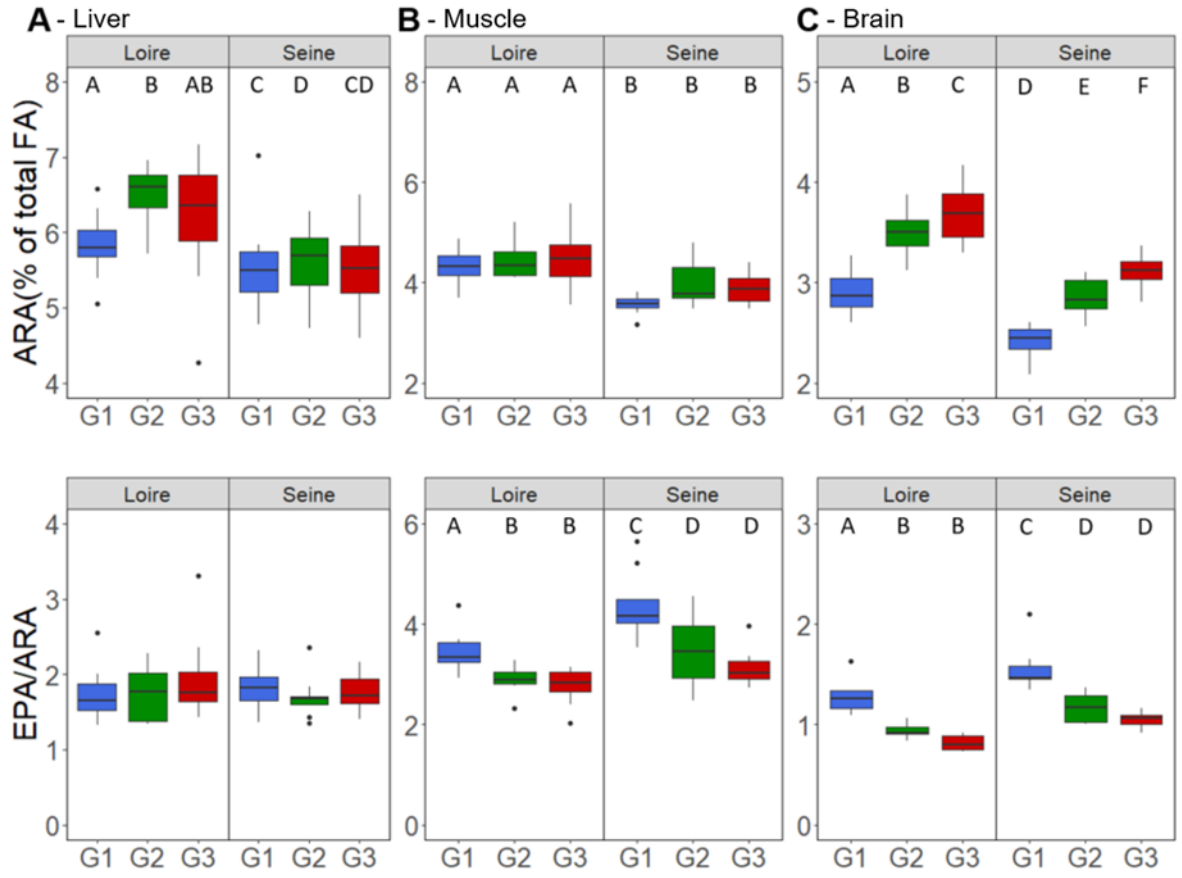

Figure 4: ARA proportions (percentage of total FA) and EPA/ARA ratios for liver (A), muscle (B) and brain (C) of juvenile European sea bass from Seine and Loire. Different letters indicate significant differences. Letters in lowercase represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc) and uppercase letters represent a significant difference between either stage or estuary without significant interaction. Significance was accepted at $p < 0.05$. Liver: Loire G1 (n=18), Loire G2 (n=11), Loire G3 (n=12), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12). Muscle: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=8), Seine G2 (n=8), Seine G3 (n=8). Brain: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=7), Seine G2 (n=8), Seine G3 (n=8).

3.4. Gene expression

The *fads2* and *scd1b* gene expressions followed the same pattern in all groups (Fig. 5A, B). Their highest expressions were measured in G1 from Seine, while their lowest expressions were measured

in the G1 and G2 from Loire, as well as in the G3 from Seine. Negative correlation between DHA and *fads2* expression was observed for the G1 Seine group as well as significant correlation between FA of the n-3 series (Fig. S3). The *lpl* gene expression did not differ significantly among ontogenetic stages, but significantly differed between the two sites, and were being globally lower in the Loire groups than in the Seine groups (Fig. 5C). The *sdhc* and *plag12b* gene expression were not significantly different between groups.

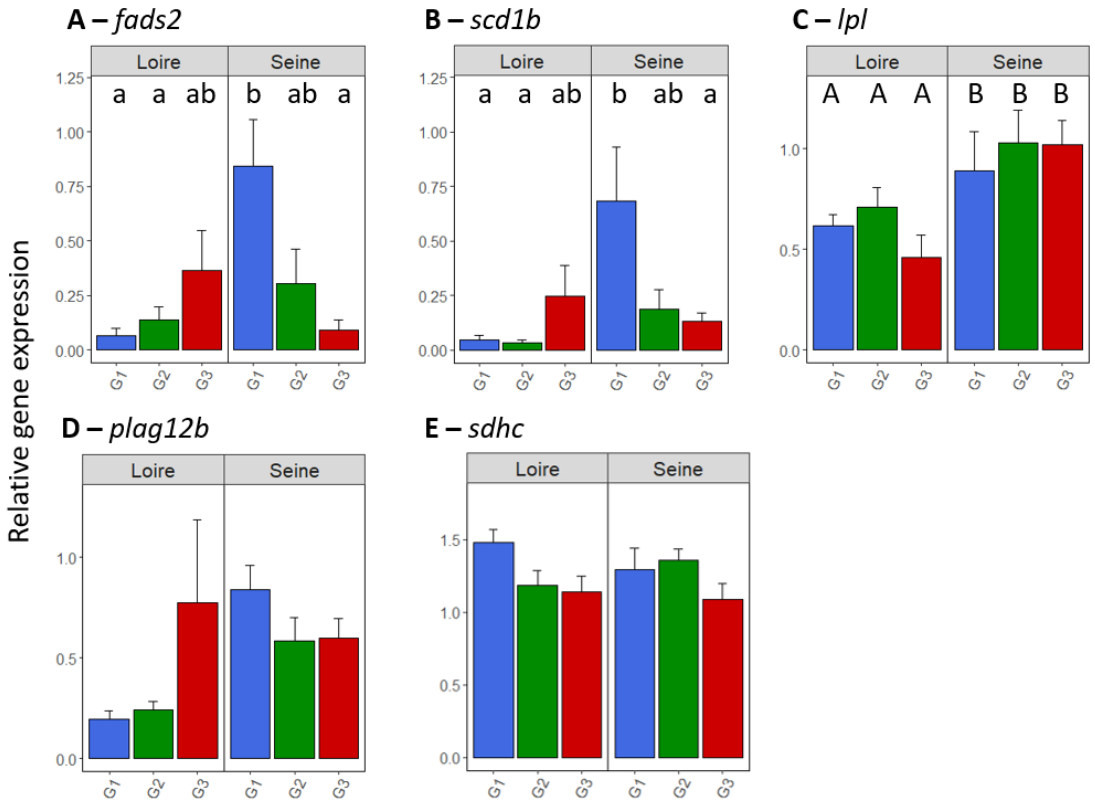

Figure 5: Relative expression of genes coding for enzymes involved in lipid metabolism in the liver of juvenile European sea bass from Loire and Seine, according to their ontogenetic stage (G1, G2, G3, corresponding to first, second and third years old, respectively). The *fads2,* fatty acid desaturase 2 (A), *lpl,* lipoprotein lipase (B), *plag12b*, group XIIB secretory phospholipase A2 (C), *scd1b*, stearoyl-CoA desaturase 1b (D), *sdhc*, succinate dehydrogenase cytochrome b560 subunit (E). Different letters indicate significant differences among groups. Letters in lowercase represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc), and uppercase letters represent a significant difference between either stage or estuary without significant interaction. Significance was accepted at $p < 0.05$. Loire G1 (n=15), Loire G2 (n=12), Loire G3 (n=11), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12).

4. Discussion

The present study aimed to explore the distribution and molecular modulation of fatty acid in different tissues of juvenile European sea bass through the ontogenetic stages from two different estuarine environments. We highlighted that PL and NL FA were differently distributed across the liver, muscle and brain. Focusing on membrane lipids (PL), FA profiles were influenced by

ontogenetic stage and estuary of origin in muscle and in liver while only ontogenetic variations were observed in the brain. Essential FA (DHA, EPA, and ARA) proportions were also influenced by estuarine environment and ontogenetic stages. At molecular level, the activation of LC PUFA biosynthetic pathways was the highest in the group that had the lowest DHA proportions in liver and muscle (Seine G1).

4.1. FA profile of juvenile European sea bass

4.1.1. SFA, MUFA and PUFA distribution in NL and PL

Different trends were observed in FA proportions among tissues and between the two lipid fractions. Our results showed that the main SFA, MUFA and PUFA were palmitic acid (16:0), oleic acid (18:1n-9) and DHA (22:6n-3), respectively, whatever the fraction or the organ considered. Muscle and liver PL had higher proportions of PUFA than brain PL, particularly in DHA (22:6n-3) and EPA (20:5n-3). This is in accordance with previous studies reporting that white muscle specifically retains DHA from the diet through selective incorporation mechanisms (Bell et al., 2001; Mourente and Bell, 2006). However, the brain is also a tissue that is known to selectively retain DHA in order to preserve cognitive functions (Lauritzen et al., 2001). Thus, the higher proportions of PUFA in the muscle and liver than in the brain might suggest these FAs are not limitant for fish. They are sufficiently retained in brain membranes, though they accumulate in muscle and liver to be likely further used as an energy source (Betancor et al., 2021; Hong et al., 2014). Compared to the muscle and liver, the brain exhibited a specific FA composition with high levels of MUFA (especially 18:1n-9 and nervonic acid, 24:1n-9) and low levels of EPA, which is consistent with previous results on the same species in controlled conditions (Granafei et al., 2017; Skalli et al., 2006). In NL the higher 16:0 and 18:1n-9 proportions in NL compared to PL, especially in liver, could be related to energy storage. These FA are produced by lipogenic activity and are known to be preferentially used as substrates for energy through □-oxidation (Bell et al., 2004; Henderson and Sargent, 1985; Sargent et al., 2003; Tocher, 2003). The liver is also the major lipid storing site in lean marine fish such as sea bass, thus explaining the storage of these FA in this organ (Mourente and Bell, 2006).

4.1.2. FA profiles of PL according to estuaries and ontogenetic stage

The muscle tissue also showed variation among both ontogenetic stages and estuaries. This might indicate specific dietary preferences or prey availability, reflecting the distinct trophic systems of each estuary (Darnaude et al., 2004). Indeed, some FAs are considered as trophic markers and can be used to identify certain primary producers (Dalsgaard et al., 2003). Seine G1 FA profiles were distinguished by high proportions of 16:1n-7, a diatom FA trophic marker (Cañavate et al., 2019) and Loire G1 FA profile were distinguished itself by high proportions of 18:2n-6, a cryptophyte trophic

marker (Viso and Marty, 1993). This could indicate different primary production between each estuary. Additionally, the differences in FA composition observed between ontogenetic stages may be indicative of distinct life stages dependent metabolic requirements, as younger fish typically have higher growth rates and metabolic demands (Jobling, 1995). It has been suggested that the phospholipid requirements would decrease with development from larvae to juveniles (Tocher et al., 2008) and could have a repercussion on the membranes of juveniles from different ages. Also, the G3 being closer to reproduction, this might have enhanced needs for essential FA (Izquierdo et al., 2001). Interestingly, no discernible pattern of PL FA composition was found in the liver among the groups (G1, G2, and G3) for both estuaries, even if some groups were statistically different from others. As previously discussed, the liver is a primary site for lipid metabolism, and it tends to have a dynamic fatty acid profile reflective of both diet and metabolic regulation (Tocher, 2003). This absence of pattern may suggest a quick turn-over of the overall FA profile in the liver (Mohan et al., 2016). Despite the not significant results, brain FA composition showed a trend with EPA discriminating the G1 stage and ARA discriminating the G3 in both estuaries. This could result from trophic difference between ontogenetic groups, as brain FA composition of fish has been proven to be modulated by dietary FA in *D. labrax* (Pagliarani et al., 1986) and gilthead sea bream (*Sparus aurata*, Carvalho et al., 2022) or from different needs for brain development.

4.2. Spatial and ontogenetic variability of Essential Fatty Acids (EFA)

The tissue-specific DHA proportion measured in wild fish in the present study are in accordance with what is usually observed experimentally in fish from aquaculture when fed a controlled diet (Skalli et al., 2006). The lower DHA proportions in fish liver from Loire G3 could also indicate a reallocation of the DHA from the liver to the growing reproductive organs, as it has previously been shown in zebrafish *Danio rerio* (Zhu et al., 2019). This hypothesis is supported by the greater length of the G3 fish from Loire than those from Seine, likely indicating a closer sexual maturity. It has been reported to happen at a minimum of 32 cm for males in the Atlantic waters (Pawson and Pickett, 1996). The increasing DHA content from Seine G1 to Seine G3 in muscle, concomitant with decreased EPA content, could be explained by a shift of diet from zooplankton to diverse epibenthic fauna through ontogenetic stages(Aprahamian and Barr, 1985; Pickett and Pawson, 1994). Fish as a prey are richer in DHA than invertebrates (*e.g Mysidacea*) that are richer in EPA (Daly et al., 2010), the older and bigger fish would then incorporate more DHA in their muscle tissue where selective retention happens. Interestingly, the lowest DHA proportion measured in the Seine G1 group was associated with higher relative *fads2* and *scd1b* gene expressions in liver (Fig. S3). While the biosynthesis of LC-PUFA (including DHA, EPA and ARA) has been shown to be very limited in most vertebrates (Tocher et al., 2019), the upregulation of the *fads2* gene expression has been evidenced in controlled

conditions in which fish, including sea bass, were fed low quantities of LC-PUFA (Geay et al., 2010b; González-Rovira et al., 2009; Vagner et al., 2009, 2007a). We could thus hypothesize that the lower DHA content associated with the higher *fads2* expression measured in the Seine G1 group may be related to (i) a lower LC-PUFA in their diet, combined to (ii) higher DHA demand due to a higher cellular turn-over in this growing ontogenetic stage. Additionally, the mean DHA proportions measured in the liver and muscle of Seine G1 were slightly lower than those reported by Skalli et al., (2006) (28% vs 30%, 23% vs 24%, respectively) in the same species, from aquaculture, and experimentally fed with a low PUFA diet (0.4% EPA+DHA on dry matter basis). The needs have been experimentally established at 0.7% EPA+DHA on dry matter basis for sea bass juveniles (Skalli and Robin, 2004). Taken together, these results would support the hypothesis of a dietary limitation in DHA for Seine G1 which would not match to their requirement at this age. Below the threshold of 0.7% EPA+DHA, growth of the juvenile was negatively affected (Skalli and Robin, 2004). However, it is important to consider that this study was conducted in experimental conditions with a goal of optimizing the aquafeed costs for European sea bass farming. Environmental conditions, such as salinity or temperature, can also influence *fads2* expression in teleost fish (for review, see Vagner and Santigosa, 2011), making it difficult to disentangle the reasons underlying the higher *fads2* expression observed in the G1 from Seine. Remarkably, the brain DHA proportion in Seine G1 was maintained as high as in all the other groups, showing likely the preservation of cognitive functions. Despite that, the effects of a possible DHA dietary limitation for this group in the environmental context should not be overlooked as it could impact growth, performances and ability to cope with changing conditions (Bou et al., 2017). The DHA content in the brain was lower in Loire G2 compared to Loire G3 and all ontogenetic stages in Seine. However, this difference was not seen in the muscle or liver. Given that the brain is a stable tissue, as noted by (Carvalho et al., 2022) and (Hong et al., 2014), it's difficult to attribute this difference to a lack of DHA in the diet. Instead, (Skalli et al., 2006) found that *D. labrax* raised at 29°C had lower DHA content than those at 22°C, suggesting temperature affects the FA composition more than diet does.

As observed for DHA, EPA and ARA proportions also displayed a tissue-specific response and differed between estuaries and between ontogenetic stages. However as the pattern observed in the liver was inversely related to that of DHA, this suggests some modulation by trophic interactions or intense metabolic hepatic activity. Similarly, the distribution of EPA in the muscle was opposite to that of DHA, with the youngest individuals displaying the highest levels of EPA. EPA was shown to be an expendable PUFA for the brain in fish unlike DHA who make up for the majority of brain membrane FA and have a proven role in neural functions (Emery et al., 2016; Trushenski et al., 2012). Contrary to EPA, the ARA proportions in brain membranes increased with age and were higher in

Loire compared to Seine. Both EPA and ARA are precursors in the production of eicosanoids (prostaglandins, leukotrienes and thromboxanes) (Gómez-Abellán and Sepulcre, 2016). Leukotrienes play vital roles in the immune response of vertebrates, and can be produced by every tissue (Rowley et al., 1995; Sargent et al., 1999). Prostaglandins are of physiological importance for respiratory functions (McKenzie et al., 1998) and osmoregulation (Ruggeri and Thoroughgood, 1985). ARA has also been proven to reduce stress in fish through the modulation of cortisol, a hormone linked to stress regulation and modulated by prostaglandins (Koven et al., 2003; Van Anholt et al., 2004). Lebigre et al., (2022) analyzed the cortisol content in the scales of juvenile European sea bass from the same cohorts (including G1, G2 and G3) in both Seine and Loire estuaries. They found a peak of cortisol in 2019, the year in which the fish were sampled in the present study, compared to other years (2017 and 2018). They also reported that cortisol concentration increased with the ontogenetic stage. The authors underlined the fact that chronic stress has a negative effect on the growth of the fish. Then, the lower values of weight and size reported in fish from the Seine estuary could partly be explained by a higher chronic stress may be due to higher pollution level or strong salinity changes for example. All together, these results suggest that increasing ARA content in the brain with life stages could be induced by the selective retention of this FA to produce eicosanoids and cope with environmental and anthropogenic stressors.

5. Conclusions

In conclusion, our study addresses critical gaps in understanding the variability of FA composition in wild European seabass. Our findings confirm the tissue-specific responses in FA distribution, with the brain exhibiting a unique FA composition compared to muscle and liver. The influence of both estuary and ontogenetic stage on membrane FA profiles, especially essential FA, highlights the complex interaction of factors that modulate FA composition. Notably, our results indicate a potential shift in prey selection as fish grow, impacting FA composition. The molecular activation of the LC PUFA synthesis pathway, particularly associated with lower DHA levels in the liver, suggests the ability of wild European sea bass to modulate their FA biosynthetic pathways at the molecular level in response to a dietary deficiency in the natural environment. Future investigations should delve into potential metabolic and behavioral implications of DHA depletion during the crucial juvenile life stage, aligning with our hypotheses on distinct organ profiles, ontogenetic influences, and the relationship between LC PUFA and lipid metabolism.

6. Competing interests

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

7. CRediT authorship contribution statement

Mickaël Peron: Conceptualization, Formal analysis, Investigation, Data curation, Writing- Original draft, Visualization. **Romain Gonzalvez:** Validation, Formal analysis, Investigation, Data curation, Writing – Review & Editing. **Sarah Hue**: Investigation. **Philippe Soudant**: Conceptualization, Validation, Writing – Review & Editing, Supervision. **Fabienne Le Grand:** Conceptualization, Validation, Writing – Review & Editing, Supervision. **David Mazurais:** Conceptualization, Validation, Writing – Review & Editing, Supervision. **Marie Vagner:** Conceptualization, Methodology, Validation, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition.

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