Marine Environmental Research 197 (2024) 106456

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

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

Mickaël Péron [a,*], Romain Gonzalvez [a], Sarah Hue [b], Philippe Soudant [a], Fabienne Le Grand [a], David Mazurais [a], Marie Vagner [a]

[a] Univ Brest, CNRS, IRD, Ifremer, UMR 6539, LEMAR, Plouzané, France
[b] UMR-I 02 SEBIO - Stress Environnementaux et BIOsurveillance des milieux aquatiques, Université du Havre Normandie, France

ARTICLE INFO

Keywords:
Lipid composition
Fish
Euryhaline DHA
EPA
ARA
LC-PUFA
Gene expression
FADS2
Molecular biosynthesis pathways

ABSTRACT

This study evaluated how estuary of origin and ontogenetic stage influence the fatty acid (FA) composition in the tissues of wild European sea bass juvenile. We evidenced tissue-specific patterns, with the brain exhibiting a distinct FA composition from the liver and muscle. Ontogenetic stage and estuary influenced the general FA profile, and particularly the essential FA (EFA) like docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) in all tissues. The data also revealed the ability of wild sea bass to modulate, at the molecular level, FA biosynthesis pathways and suggest a potential dietary DHA limitation in the natural environment. The distribution of FA within tissues might reflect shifts in diet, metabolic demands, or adaptations to environmental conditions. This study provides insights about FA dynamics in euryhaline fish during juvenile life stage, improving our understanding of the metabolism need and EFA trophic availability in a changing environment.

1. Introduction

Long-chain polyunsaturated fatty acids (≥20 carbon atoms, LC-PUFA), particularly eicosapentaenoic acid (EPA; 20:5n-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 (Sargent et al., 2003). EPA and ARA also 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 compositions 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 (Tocher et al., 2008). 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 is 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., 2007a, 2009).

Recent environmental changes of temperature, ocean pH, and

* Corresponding author.
E-mail address: Mickael.peron1@gmail.com (M. Péron).

https://doi.org/10.1016/j.marenvres.2024.106456
Received 4 October 2023; Received in revised form 25 January 2024; Accepted 14 March 2024
Available online 21 March 2024

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 have profound cascading effects on fish physiology, including reduced growth, altered energy metabolism, reduced immune function, and impaired reproductive success (Bell and Koppe, 2010; Schmitz and Ecker, 2008; Vagner et al., 2014, 2015, 2019). 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 FA 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 the 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 the 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 wild fish to changing environmental conditions, with a focus on the role of FA in this process. Specifically, we hypothesize 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 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 the 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. Gallbladder was removed from the liver. About 200 mg of muscle were taken on the left side of the fish, dorsally from the lateral line and just behind the head. 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 (K_n) (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 K_n 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 neutral (NL) and polar (PL) fractions 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 × 4 mm, silica gel 60A 63–200 μm rehydrated using 6% H_2O (70–230 mesh)). NL were eluted using 10 mL of a mixture of chloroform/methanol (98:2, v/v) and PL were then eluted using 20 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 min 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 × 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 (μg 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 *b*560 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 comparison was realized using a PERMANOVA followed by pairwise tests (package *vegan*, *pairwise adonis* function) to test for significant differences 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, and 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: $X \sim Estuary + Stage$ 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 K_n, however, remained constant across developmental stages for both estuaries and no significant differences were observed in relation to either the stage or estuary.

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, MUFA, PUFA) were relatively similar between both tissues. A balanced distribution among FA families was observed in muscle, with relatively close concentrations: SFA (30%),

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 (the Seine and the 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	–	–	–

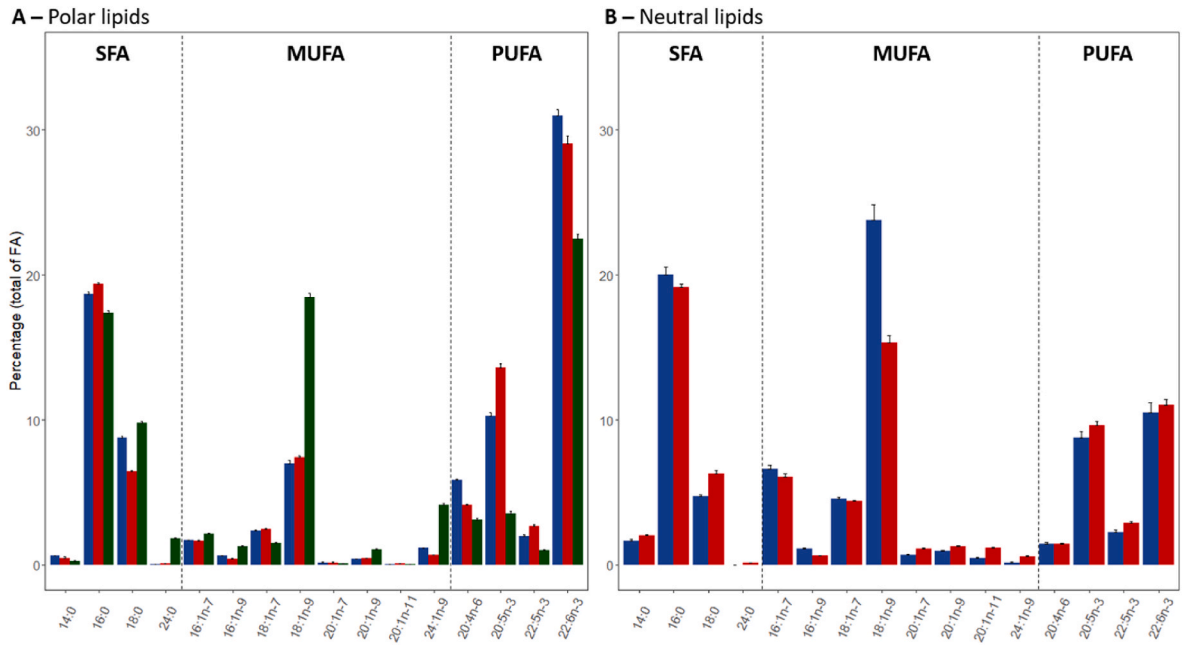

Fig. 1. Proportions of fatty acids (mass percentage of total FA) in polar lipids (A) and neutral lipids (B) in the liver (blue, n = 75), muscle (red, n = 50) and brain (green, n = 49) of juvenile European sea bass from combined locations of the Seine and the Loire and all ontogenetic stages. Only FA 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.

MUFA (32%) and PUFA (33%), with 16:0, 18:1n-9, EPA and DHA as major FA in both tissues. Liver had the highest concentrations of MUFA (40%; Fig. S2 B), with 18:1n-9 being the major FA (Fig. 1B).

The inertia of FA composition in fish PL, according to organs, ontogenetic stages and sampling locations 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, Tables S3 and 4). In the Seine estuary, the G1 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, Tables S3 and S4). The Seine G1 group was different from all the other groups except from the 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 the Seine tending to be characterized by 16:0 and 18:0, and the Loire by 22:5n-6 and ARA (20:4n-6). Brain FA composition in PL (Fig. 2C) was influenced by an interactive effect between site and ontogenetic stages, with the Seine G2 group being different from Loire G1 and Loire G2 (PERMANOVA, Tables S3 and 4). The G1 seemed to be distinguished by EPA and DPA (22:5n-3) and the G3 by ARA and 16:1n-9.

3.3. FA proportions and ratios between ontogenetic stages 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 the Loire, while it tended to increase in the Seine. In muscle (Fig. 3 B), DHA significantly increased with the ontogenetic stage in the Seine, while it remained similar in all ontogenetic groups in the 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 the Seine. The G2 in the Loire had a lower DHA content than the G3 in the Loire and was lower than all ontogenetic stages of the Seine.

3.3.2. EPA proportions

For the liver (Fig. 3A) in the 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 the Seine groups. In muscle and brain (Fig. 3B and 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.

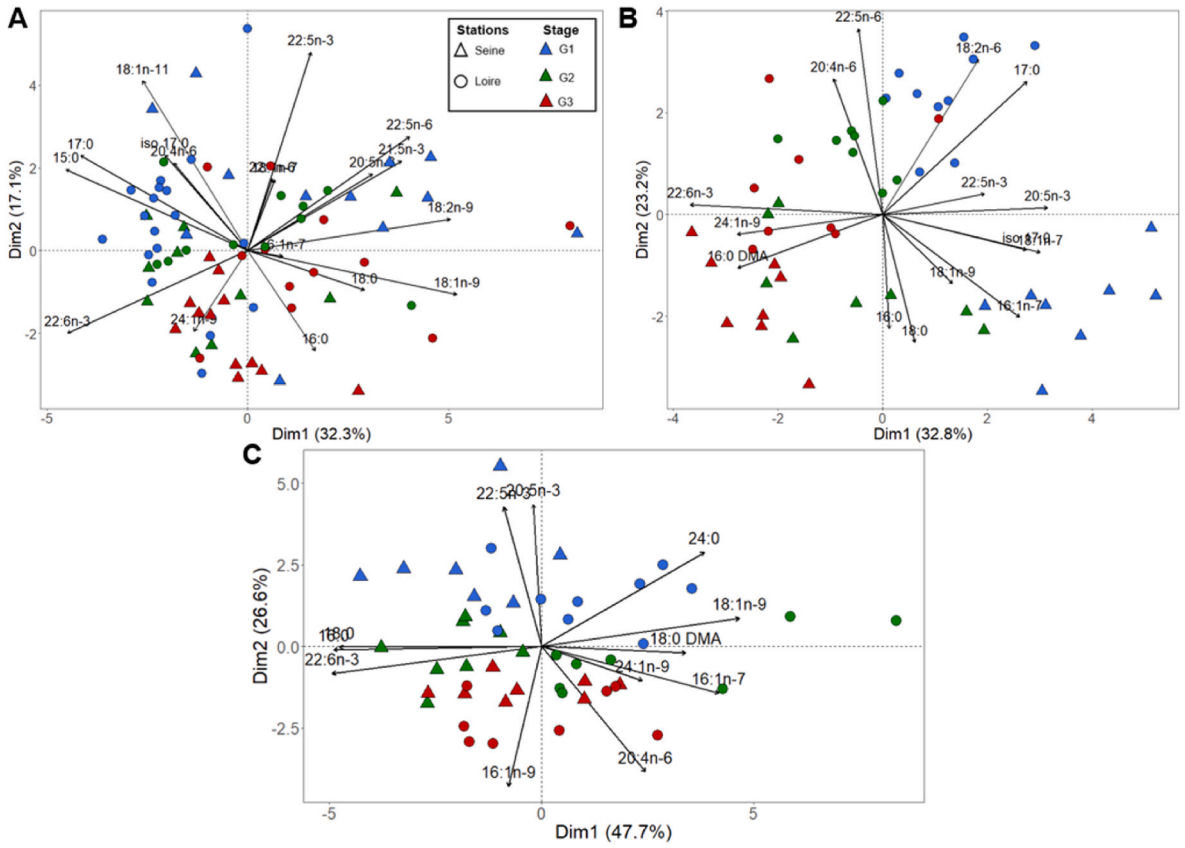

Fig. 2. Principal Component Analysis (PCA) of polar lipid fatty acids in liver (A), muscle (B) and brain (C) of 1 (G1), 2 (G2) or 3 (G3) years old juvenile European sea bass from the Seine and the Loire. Only FA that account for >80% of the contribution of dissimilarity between groups are shown (SIMPER 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).

3.3.3. 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 the Loire it was lower for G1 and G2 compared to G3.

3.3.4. ARA proportions

In all tissues, ARA proportions were higher in the Loire than in the Seine. In the liver (Fig. 4A), ARA proportions were higher in G2 than in G1 in both estuaries. In muscle (Fig. 4B), it did not differ between ontogenetic stages in both estuaries, while in the brain (Fig. 4C), it increased consistently with stage 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 and C), the EPA/ARA ratio was higher in the Seine than in the Loire and G1 had a higher ratio than G2 and G3.

3.4. Gene expression

The *fads2* and *scd1b* gene expressions followed the same pattern in all groups (Fig. 5A and B). Their highest expressions were measured in G1 from the Seine, while their lowest expressions were measured in the G1 and G2 from the Loire, as well as in the G3 from the Seine. A negative correlation between DHA and *fads2* expression was observed for the G1 Seine group, as well as a 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 estuaries, and was globally lower in the Loire groups than in the Seine groups (Fig. 5C). The *sdhc* and *plag12b* gene expressions were not significantly different between groups.

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 evidenced that PL and NL FA were differentially distributed between liver, muscle and brain. Focusing on membrane lipids (PL), FA profiles were influenced by ontogenetic stage and estuary in the muscle and in the 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 stage. At the molecular level, the activation of LC-PUFA biosynthetic pathways was the highest in the group coupled with the lowest DHA proportions in the liver and in the 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

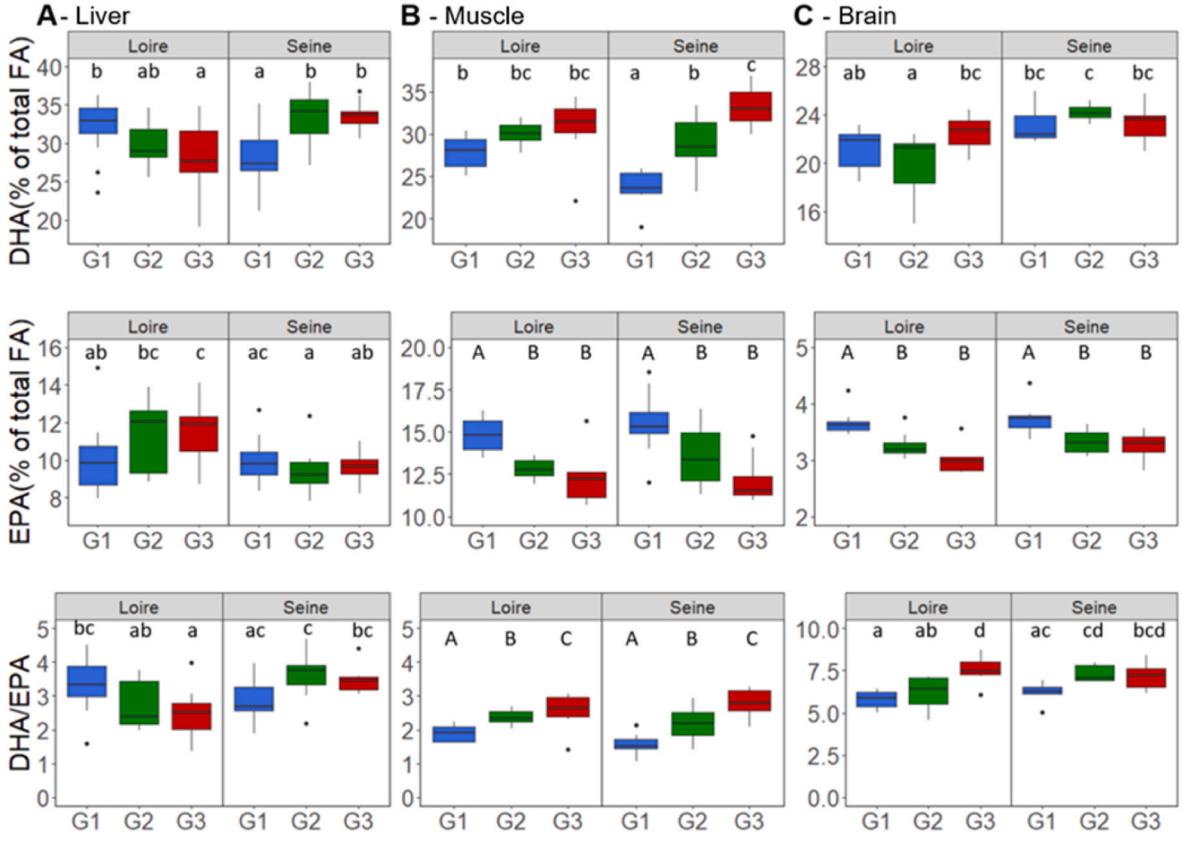

Fig. 3. Proportions of DHA and EPA (percentage of total FA) and DHA/EPA ratio in the polar lipids of liver (A), muscle (B) and brain (C) of juvenile European sea bass from the Seine and the Loire estuaries. Different letters within a plot indicate significant differences. Lowercase letters represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc). Capital letters indicate a significant difference among either stages or estuaries 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).

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 brain functions (Lauritzen et al., 2001). Thus, the higher proportions of PUFA in the muscle and liver than in the brain might suggest that these FA are not limitant for fish. The PUFA 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 higher levels of MUFA (especially 18:1n-9 and nervonic acid, 24:1n-9) and lower levels of EPA, which is consistent with previous results obtained on the same species in controlled conditions (Granafei et al., 2017; Skalli et al., 2006). The higher 16:0 and 18:1n-9 proportions in NL compared to PL, especially in liver, could be related to energy storage function. These FA are produced by lipogenic activity and are known to be preferentially used as substrates for energy through β-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 stages

The muscle tissue FA content 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 FA are considered as trophic markers and can be used to identify certain primary producers (Dalsgaard et al., 2003). Seine G1 FA profile was distinguished by high proportions of 16:1n-7, a diatom FA trophic marker (Cañavate et al., 2019) and Loire G1 FA profile was distinguished itself by high proportions of 18:2n-6, a cryptophyte trophic marker (Viso and Marty, 1993). This could indicate different primary productions between estuaries. Additionally, the differences in FA composition observed between ontogenetic stages may be indicative of different life stage 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 membrane composition of juveniles from different ages. Also, the G3 being closer to reproduction, this might have enhanced needs for essential FA (Izquierdo et al., 2001). PL FA composition in the liver of the groups (G1, G2, and G3) for both estuaries did not reveal discernible patterns, although some groups were statistically different from others. As previously discussed, the liver is a primary organ for lipid metabolism, and it tends to have a dynamic fatty acid profile, reflective of both diet and metabolic regulation (Tocher, 2003). The absence of pattern may suggest a quick turn-over of the overall FA profile in the liver (Mohan et al., 2016). Despite not significant differences, 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.

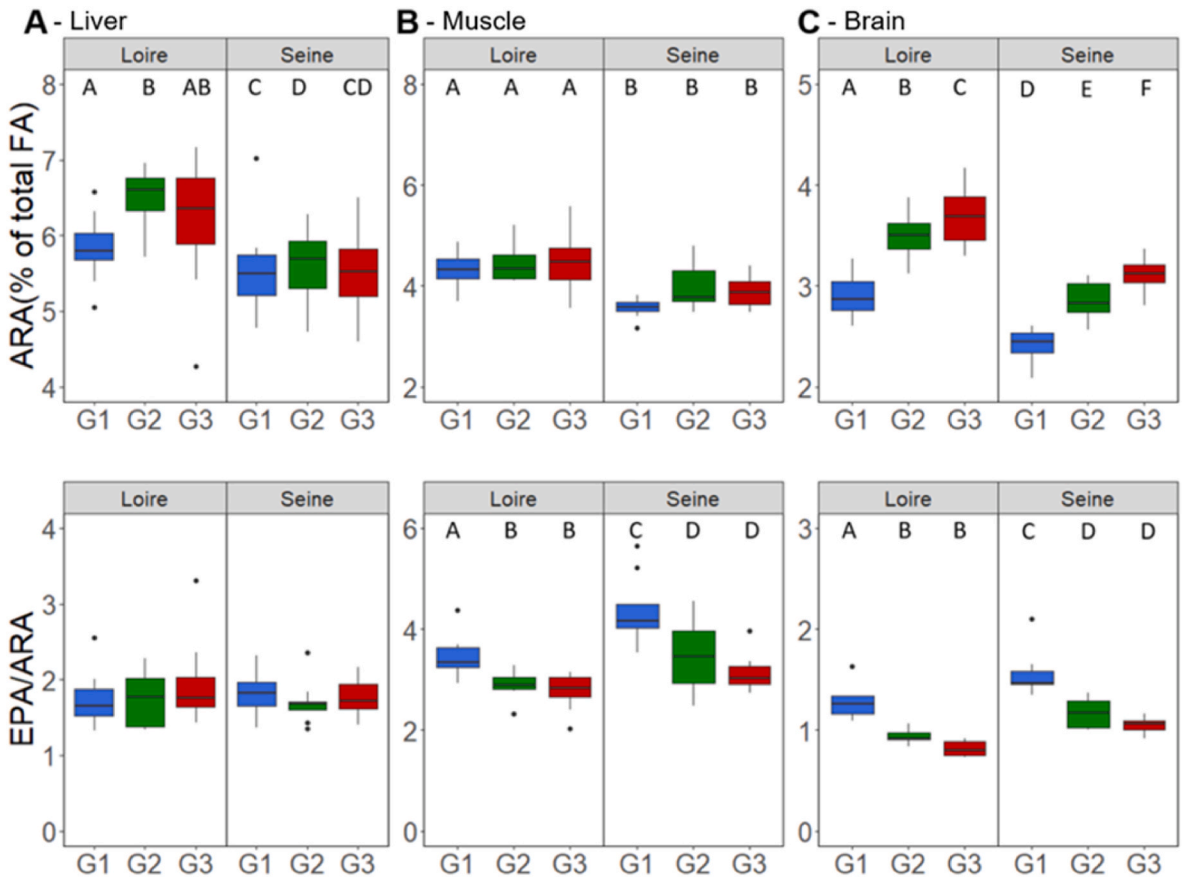

Fig. 4. ARA proportions (percentage of total FA) and EPA/ARA ratios in the polar lipids of liver (A), muscle (B) and brain (C) of juvenile European sea bass from the Seine and the Loire estuaries. Different letters within a plot indicate significant differences. Lowercase letters represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc). Capital letters indicate a significant difference among either stages or estuaries 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).

4.2. Spatial and ontogenetic variability of essential fatty acids

The tissue-specific DHA proportions measured in wild sea bass in the present study are in accordance with what is usually observed experimentally in farmed fish fed a controlled diet (Skalli et al., 2006). The lower DHA proportions in the G3 sea bass liver from the Loire 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 the Loire than those from the Seine, likely indicating a more advanced sexual maturity. The sexual maturity of *D. labrax* 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 proportions 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., 2007a, 2009). We could thus hypothesize that the lower DHA proportions 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 average DHA proportions measured in the liver and in the muscle of G1 from the Seine were slightly lower than those reported by Skalli et al. (2006) (28% vs 30%, 23% vs 24%, respectively) in the same species, from aquaculture origin, and experimentally fed with a low PUFA diet (0.4% EPA + DHA on dry matter basis). The EPA and DHA dietary requirements have been experimentally established at 0.7% EPA + DHA on dry matter (DM) basis for sea bass juveniles (Skalli and Robin, 2004). Taken together, these results would support the hypothesis of a dietary limitation in DHA for the G1 from the Seine which would not meet their requirement at this age. Below the threshold of 0.7% EPA + DHA DM, growth of the juvenile sea bass 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 the Seine. Remarkably, the brain DHA proportion in Seine G1 was maintained as high as in all the other groups, showing the likely preservation of brain functions. However, the effects of a possible DHA dietary limitation for this group in the environmental context should not be overlooked as it could impact growth performance and the ability of fish to

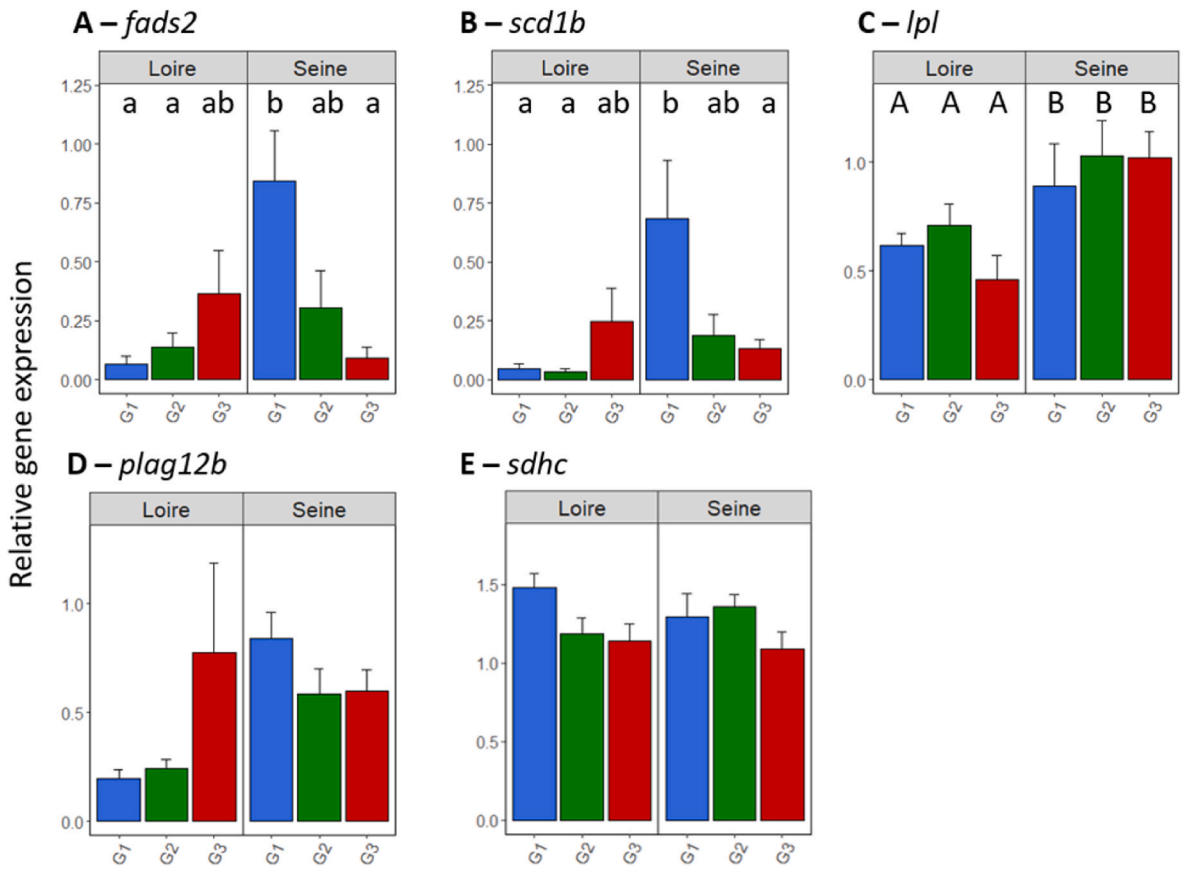

Fig. 5. Relative expression of genes coding for enzymes involved in lipid metabolism in the liver of juvenile European sea bass from the Loire and the Seine estuaries, according to their ontogenetic stage (G1, G2, G3, corresponding to first, second and third years old, respectively). The *fads2,* fatty 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 *b*560 subunit (E). Different letters indicate significant differences among groups. Lowercase letters represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc). Capital letters indicate a significant difference among either stages or estuaries 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).

cope with changing environmental conditions (Bou et al., 2017). The DHA proportions in the brain was lower in the G2 from the Loire compared to the G3 from the Loire and to all ontogenetic stages in Seine. Yet, this difference was not seen in the muscle or the liver. Given that the brain is a stable tissue, as noted by (Carvalho et al., 2022; 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 also affects the FA composition.

As observed for DHA, EPA and ARA proportions also displayed a tissue-specific distribution and differed between estuaries and between ontogenetic stages. However as the pattern observed in the liver was inversely related to that of DHA, it 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 has been shown to be an expendable PUFA for the brain in fish unlike DHA which makes up for the majority of brain membrane FA and have a proven role in brain 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 the Loire compared to the Seine. Both EPA and ARA are precursors for 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 the Seine and the 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 possibly due to higher pollution level or consequent 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 sea bass. Our findings reveal the tissue-specific FA distribution, with the brain exhibiting a distinct FA composition compared to muscle and liver. Estuarine environments and the ontogenetic stage significantly influence membrane FA, particularly EFA. Notably, our results indicate a potential shift in prey selection as fish grows, influencing FA composition in the end. The

molecular activation of the LC-PUFA synthesis pathway, particularly associated with lower DHA levels in the liver, suggests an ability of wild European sea bass to compensate, to some extent, a dietary limitation in its natural environment. Future investigations should delve into metabolic and behavioral implications of DHA limitation during the juvenile life stage.

CRediT authorship contribution statement

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

Declaration of competing interest

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.

Data availability

Data will be made available on request.

Acknowledgments

We thank Mickaël Drogou, Stéphane Martin, Loïc Le Ru and Christophe Lebigre for their help collecting fish during the NOURDEM campaign. We are grateful to Karine Salin and Eric Dabas for their help with onboard fish dissections and sampling. We thank Antoine Bideau and Lauriane Madec for their help with the lipid and molecular analyses. This work was supported by ISblue project, Interdisciplinary graduate school for the blue planet (ANR-17-EURE-0015) and co-funded by a grant from the French government under the program "Investissements d'Avenir", by the French National program EC2CO (Ecosphère Continentale et Côtière), and the Scientific Council of the European Institute of Marine Sciences.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2024.106456.

References

Aprahamian, M.W., Barr, C.D., 1985. The growth, abundance and diet of O-group sea bass, Dicentrarchus labrax, from the severn estuary. J. Mar. Biol. Assoc. U. K. 65, 169–180. https://doi.org/10.1017/S0025315400060884.

Beck, M.W., Heck, K.L., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates: a better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. Bioscience 51, 633–641. https://doi.org/10.1641/0006-3568(2001)051[0633:TICAMO]2.0.CO;2.

Bell, J.G., Henderson, R.J., Tocher, D.R., Sargent, J.R., 2004. Replacement of dietary fish oil with increasing levels of linseed oil: modification of flesh fatty acid compositions in Atlantic salmon (Salmo salar) using a fish oil finishing diet. Lipids 39, 223–232. https://doi.org/10.1007/s11745-004-1223-5.

Bell, J.G., Koppe, W., 2010. Lipids in aquafeeds. Fish oil replacement and alternative lipid sources in aquaculture feeds 1, 21–59.

Bell, J.G., McEvoy, J., Tocher, D.R., McGhee, F., Campbell, P., Sargent, J.R., 2001. Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (Salmo salar) affects tissue lipid compositions and hepatocyte fatty acid metabolism. J. Nutr. 131, 1535–1543.

Betancor, M.B., MacEwan, A., Sprague, M., Gong, X., Montero, D., Han, L., Napier, J.A., Norambuena, F., Izquierdo, M., Tocher, D.R., 2021. Oil from transgenic Camelina sativa as a source of EPA and DHA in feed for European sea bass (Dicentrarchus labrax L.). Aquaculture 530, 735759. https://doi.org/10.1016/j.aquaculture.2020.735759.

Bhouri, A.M., Bouhlel, I., Chouba, L., Hammami, M., Cafsi, M.E., Chaouch, A., 2010. Total lipid content, fatty acid and mineral compositions of muscles and liver in wild and farmed sea bass (Dicentrarchus labrax). AJFS 4, 522–530. https://doi.org/10.5897/AJFS.9000301.

Blaber, S.J.M., Blaber, T.G., 1980. Factors affecting the distribution of juvenile estuarine and inshore fish. J. Fish. Biol. 17, 143–162. https://doi.org/10.1111/j.1095-8649.1980.tb02749.x.

Bou, M., Berge, G.M., Baeverfjord, G., Sigholt, T., Østbye, T.-K., Romarheim, O.H., Hatlen, B., Leeuwis, R., Venegas, C., Ruyter, B., 2017. Requirements of n-3 very long-chain PUFA in Atlantic salmon (Salmo salar L): effects of different dietary levels of EPA and DHA on fish performance and tissue composition and integrity. Br. J. Nutr. 117, 30–47. https://doi.org/10.1017/S0007114516004396.

Brett, M.T., Kainz, M.J., Taipale, S.J., Seshan, H., 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. Proc. Natl. Acad. Sci. USA 106, 21197–21201. https://doi.org/10.1073/pnas.0904129106.

Calder, P.C., 2017. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem. Soc. Trans. 45, 1105–1115. https://doi.org/10.1042/BST20160474.

Cañavate, J.-P., van Bergeijk, S., Giráldez, I., González-Ortegón, E., Vílas, C., 2019. Fatty acids to quantify phytoplankton functional groups and their spatiotemporal dynamics in a highly turbid estuary. Estuar. Coast 42, 1971–1990. https://doi.org/10.1007/s12237-019-00629-8.

Carvalho, M., Montero, D., Domenici, P., Afonso, J.M., Izquierdo, M., 2022. Dietary novel oils modulate neural function and preserve locomotor response in gilthead sea bream (Sparus aurata) juveniles by regulating synthesis and contents of fatty acids in brain. Aquaculture 550, 737873. https://doi.org/10.1016/j.aquaculture.2021.737873.

Clarke, K.R., 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. 18, 117–143. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x.

Cottin, S.C., Sanders, T.A., Hall, W.L., 2011. The differential effects of EPA and DHA on cardiovascular risk factors. Proc. Nutr. Soc. 70, 215–231. https://doi.org/10.1017/S0029665111000061.

Dalsgaard, J., St John, M., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. In: Advances in Marine Biology. Academic Press, pp. 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7.

Daly, E.A., Benkwitt, C.E., Brodeur, R.D., Litz, M.N.C., Copeman, L.A., 2010. Fatty acid profiles of juvenile salmon indicate prey selection strategies in coastal marine waters. Mar. Biol. 157, 1975–1987. https://doi.org/10.1007/s00227-010-1466-9.

Darnaude, A.M., Salen-Picard, C., Polunin, N.V.C., Harmelin-Vivien, M.L., 2004. Trophodynamic linkage between river runoff and coastal fishery yield elucidated by stable isotope data in the Gulf of Lions (NW Mediterranean). Oecologia 138, 325–332. https://doi.org/10.1007/s00442-003-1457-3.

Emery, J.A., Norambuena, F., Trushenski, J., Turchini, G.M., 2016. Uncoupling EPA and DHA in fish nutrition: dietary demand is limited in atlantic salmon and effectively met by DHA alone. Lipids 51, 399–412. https://doi.org/10.1007/s11745-016-4136-y.

Fuentes, A., Fernández-Segovia, I., Serra, J.A., Barat, J.M., 2010. Comparison of wild and cultured sea bass (Dicentrarchus labrax) quality. Food Chem. 119, 1514–1518. https://doi.org/10.1016/j.foodchem.2009.09.036.

Galindo, A., Garrido, D., Monroig, Ó., Pérez, J.A., Betancor, M.B., Acosta, N.G., Kabeya, N., Marrero, M.A., Bolaños, A., Rodríguez, C., 2021. Polyunsaturated fatty acid metabolism in three fish species with different trophic level. Aquaculture 530, 735761. https://doi.org/10.1016/j.aquaculture.2020.735761.

Galloway, A.W.E., Winder, M., 2015. Partitioning the relative importance of phylogeny and environmental conditions on phytoplankton fatty acids. PLoS One 10, e0130053. https://doi.org/10.1371/journal.pone.0130053.

Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W.W.L., Howes, E.L., Joos, F., Allemand, D., Bopp, L., Cooley, S.R., Eakin, C.M., Hoegh-Guldberg, O., Kelly, R.P., Pörtner, H.-O., Rogers, A.D., Baxter, J.M., Laffoley, D., Osborn, D., Rankovic, A., Rochette, J., Sumaila, U.R., Treyer, S., Turley, C., 2015. Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios. Science 349, aac4722. https://doi.org/10.1126/science.aac4722.

Geay, F., Santigosa I Culi, E., Corporeau, C., Boudry, P., Dreano, Y., Corcos, L., Bodin, N., Vandeputte, M., Zambonino-Infante, J.L., Mazurais, D., Cahu, C.L., 2010a. Regulation of FADS2 expression and activity in European sea bass (Dicentrarchus labrax, L.) fed a vegetable diet. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 156, 237–243. https://doi.org/10.1016/j.cbpb.2010.03.008.

Geay, F., Santigosa I Culi, E., Corporeau, C., Boudry, P., Dreano, Y., Corcos, L., Bodin, N., Vandeputte, M., Zambonino-Infante, J.L., Mazurais, D., Cahu, C.L., 2010b. Regulation of FADS2 expression and activity in European sea bass (Dicentrarchus labrax, L.) fed a vegetable diet. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 156, 237–243. https://doi.org/10.1016/j.cbpb.2010.03.008.

Glencross, B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquacult. 1, 71–124. https://doi.org/10.1111/j.1753-5131.2009.01006.x.

Gómez-Abellán, V., Sepulcre, M.P., 2016. The role of prostaglandins in the regulation of fish immunity. Mol. Immunol. 69, 139–145. https://doi.org/10.1016/j.molimm.2015.09.022.

González-Rovira, A., Mourente, G., Zheng, X., Tocher, D.R., Pendón, C., 2009. Molecular and functional characterization and expression analysis of a Δ6 fatty acyl desaturase cDNA of European Sea Bass (Dicentrarchus labrax L.). Aquaculture 298, 90–100. https://doi.org/10.1016/j.aquaculture.2009.10.012.

Granafei, S., Liebisch, G., Palmisano, F., Carlucci, R., Lionetti, A., Longobardi, F., Bianco, G., Cataldi, T.R.I., 2017. Effect of storage and extraction protocols on the lipid and fatty acid profiles of Dicentrarchus labrax brain. Food Anal. Methods 10, 4003–4012. https://doi.org/10.1007/s12161-017-0967-2.

Henderson, R.J., Sargent, J.R., 1985. Chain-length specificities of mitochondrial and peroxisomal beta-oxidation of fatty acids in livers of rainbow trout (Salmo gairdneri). Comp. Biochem. Physiol. B 82, 79–85. https://doi.org/10.1016/0305-0491(85)90131-2.

Hixson, S.M., Arts, M.T., 2016. Climate warming is predicted to reduce omega-3, long-chain, polyunsaturated fatty acid production in phytoplankton. Global Change Biol. 22, 2744–2755. https://doi.org/10.1111/gcb.13295.

Hong, H., Zhou, Y., Wu, H., Luo, Y., Shen, H., 2014. Lipid content and fatty acid profile of muscle, brain and eyes of seven freshwater fish: a comparative study. JAOCS (J. Am. Oil Chem. Soc.) 91, 795–804. https://doi.org/10.1007/s11746-014-2414-5.

Izquierdo, M.S., Fernández-Palacios, H., Tacon, A.G.J., 2001. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture, Reproductive Biotechnology in Finfish Aquaculture 197, 25–42. https://doi.org/10.1016/S0044-8486(01)00581-6.

Jobling, M., 1995. Fish bioenergetics. Oceanogr. Lit. Rev. 9, 785.

Koven, W., van Anholt, R., Lutzky, S., Ben Atia, I., Nixon, O., Ron, B., Tandler, A., 2003. The effect of dietary arachidonic acid on growth, survival, and cortisol levels in different-age gilthead seabream larvae (Sparus auratus) exposed to handling or daily salinity change. Aquaculture 228, 307–320. https://doi.org/10.1016/S0044-8486(03)00317-X.

Lauritzen, L., Hansen, H.S., Jørgensen, M.H., Michaelsen, K.F., 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog. Lipid Res. 40, 1–94. https://doi.org/10.1016/s0163-7827(00)00017-5.

Le Cren, E.D., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilis). J. Anim. Ecol. 20, 201–219. https://doi.org/10.2307/1540.

Le Goff, R., Drogou, M., Lebigre, C., Woillez, M., Cornou, A.S., Berthele, O., Delaunay, D., Martin, S., Le Ru, L., Barone, H., Bouche, L., Roy, A., Le Roy, D., Huet, J., Garren, F., Edin, L., Michelet, N., Denize, S., Nguyen, P., Nfis, F., Le Roy, E., Marhic, C., Mahe, K., Weiss, J., 2022. NourDem 2019-2021. Rapport Final.

Le Grand, F., Soudant, P., Siah, A., Tremblay, R., Marty, Y., Kraffe, E., 2014. Disseminated neoplasia in the soft-shell clam mya arenaria: membrane lipid composition and functional parameters of circulating cells. Lipids 49, 807–818. https://doi.org/10.1007/s11745-014-3917-4.

Le Pape, O., Gilliers, C., Riou, P., Morin, J., Amara, R., Désaunay, Y., 2007. Convergent signs of degradation in both the capacity and the quality of an essential fish habitat: state of the Seine estuary (France) flatfish nurseries. Hydrobiologia 588, 225–229. https://doi.org/10.1007/s10750-007-0665-y.

Le Pape, O., Holley, J., Guérault, D., Désaunay, Y., 2003. Quality of coastal and estuarine essential fish habitats: estimations based on the size of juvenile common sole (Solea solea L.). Estuar. Coast Shelf Sci. 58, 793–803. https://doi.org/10.1016/S0272-7714(03)00185-9.

Lebigre, C., Woillez, M., Barone, H., Mourot, J., Drogou, M., Le Goff, R., Servili, A., Hennebert, J., Vanhomwegen, M., Aerts, J., 2022. Temporal variations in scale cortisol indicate consistent local-and broad-scale constraints in a wild marine teleost fish. Mar. Environ. Res. 182, 105783 https://doi.org/10.1016/j.marenvres.2022.105783.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Maltsev, Y., Maltseva, K., 2021. Fatty acids of microalgae: diversity and applications. Rev. Environ. Sci. Biotechnol. 20, 515–547. https://doi.org/10.1007/s11157-021-09571-3.

Mathieu-Resuge, M., Kraffe, E., Le Grand, F., Boens, A., Bideau, A., Lluch-Cota, S.E., Racotta, I.S., Schaal, G., 2019. Trophic ecology of suspension-feeding bivalves inhabiting a north-eastern Pacific coastal lagoon: comparison of different biomarkers. Mar. Environ. Res. 145, 155–163. https://doi.org/10.1016/j.marenvres.2019.02.016.

Mazurais, D., Servili, A., Le Bayon, N., Gislard, S., Madec, L., Zambonino-Infante, J.-L., 2020. Long-term exposure to near-future ocean acidification does not affect the expression of neurogenesis- and synaptic transmission-related genes in the olfactory bulb of European sea bass (Dicentrarchus labrax). J. Comp. Physiol. B 190, 161–167. https://doi.org/10.1007/s00360-019-01256-2.

McKenzie, D.J., Higgs, D.A., Dosanjh, B.S., Deacon, G., Randall, D.J., 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (Salmo salar) in seawater. Fish Physiol. Biochem. 19, 111–122. https://doi.org/10.1023/A:1007779619087.

Mejri, S.C., Tremblay, R., Audet, C., Wills, P.S., Riche, M., 2021. Essential fatty acid requirements in tropical and cold-water marine fish larvae and juveniles. Front. Mar. Sci. 8.

Ménesguen, A., Desmit, X., Dulière, V., Lacroix, G., Thouvenin, B., Thieu, V., Dussauze, M., 2018. How to avoid eutrophication in coastal seas? A new approach to derive river-specific combined nitrate and phosphate maximum concentrations. Sci. Total Environ. 628–629, 400–414. https://doi.org/10.1016/j.scitotenv.2018.02.025.

Mohan, S.D., Connelly, T.L., Harris, C.M., Dunton, K.H., McClelland, J.W., 2016. Seasonal trophic linkages in Arctic marine invertebrates assessed via fatty acids and compound-specific stable isotopes. Ecosphere 7, e01429. https://doi.org/10.1002/ecs2.1429.

Monroig, O., Tocher, D.R., Castro, L.F.C., 2018. Chapter 3 - polyunsaturated fatty acid biosynthesis and metabolism in fish. In: Burdge, G.C. (Ed.), Polyunsaturated Fatty Acid Metabolism. AOCS Press, pp. 31–60. https://doi.org/10.1016/B978-0-12-811230-4.00003-X.

Monroig, Ó., Zheng, X., Morais, S., Leaver, M.J., Taggart, J.B., Tocher, D.R., 2010. Multiple genes for functional Δ6 fatty acyl desaturases (Fad) in Atlantic salmon (Salmo salar L.): gene and cDNA characterization, functional expression, tissue distribution and nutritional regulation. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1801, 1072–1081. https://doi.org/10.1016/j.bbalip.2010.04.007.

Mourente, G., Bell, J.G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (Dicentrarchus labrax L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 145, 389–399. https://doi.org/10.1016/j.cbpb.2006.08.012.

Orban, E., Nevigato, T., Lena, G.D., Casini, I., Marzetti, A., 2003. Differentiation in the lipid quality of wild and farmed seabass (Dicentrarchus labrax) and gilthead sea bream (Sparus aurata). J. Food Sci. 68, 128–132. https://doi.org/10.1111/j.1365-2621.2003.tb14127.x.

Pagliarani, A., Pirini, M., Trigari, G., Ventrella, V., 1986. Effect of diets containing different oils on brain fatty acid composition in sea bass (Dicentrarchus labrax L.). Comp. Biochem. Physiol. B 83, 277–282. https://doi.org/10.1016/0305-0491(86)90366-4.

Pawson, M.G., Pickett, G.D., 1996. The annual pattern of condition and maturity in bass, Dicentrarchus labrax, in waters around england and wales. J. Mar. Biol. Assoc. U. K. 76, 107–125. https://doi.org/10.1017/S0025315400029040.

Pawson, M.G., Pickett, G.D., Witthames, P.R., 2000. The influence of temperature on the onset of first maturity in sea bass. J. Fish. Biol. 56, 319–327. https://doi.org/10.1111/j.1095-8649.2000.tb02109.x.

Pérez-Ruzafa, A., Marcos, C., 2014. Biology of European Sea Bass. CRC Press.

Pickett, G.D., Pawson, M.G., 1994. Sea Bass: Biology. Springer Science & Business Media.

Poloczanska, E.S., Brown, C.J., Sydeman, W.J., Kiessling, W., Schoeman, D.S., Moore, P. J., Brander, K., Bruno, J.F., Buckley, L.B., Burrows, M.T., Duarte, C.M., Halpern, B.S., Holding, J., Kappel, C.V., O'Connor, M.I., Pandolfi, J.M., Parmesan, C., Schwing, F., Thompson, S.A., Richardson, A.J., 2013. Global imprint of climate change on marine life. Nat. Clim. Change 3, 919–925. https://doi.org/10.1038/nclimate1958.

Pörtner, H.O., Roberts, D.C., Poloczanska, E.S., Mintenbeck, K., Tignor, M., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., 2022. IPCC, 2022: summary for policymakers. In: Pörtner, H.O., Roberts, D.C., Poloczanska, E.S., Mintenbeck, K., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., Rama, B. (Eds.), Cambridge University Pres, pp. 3–33. Cambridge, UK and New York, NY, US.

Rimoldi, S., Benedito-Palos, L., Terova, G., Pérez-Sánchez, J., 2016. Wide-targeted gene expression infers tissue-specific molecular signatures of lipid metabolism in fed and fasted fish. Rev. Fish Biol. Fish. 26, 93–108. https://doi.org/10.1007/s11160-015-9408-8.

Rowley, A.F., Knight, J., Lloyd-Evans, P., Holland, J.W., Vickers, P.J., 1995. Eicosanoids and their role in immune modulation in fish—a brief overview. Fish Shellfish Immunol. 5, 549–567. https://doi.org/10.1016/S1050-4648(95)80041-7.

Ruggeri, B., Thoroughgood, C., 1985. Prostaglandins in aquatic fauna: a comprehensive review. Mar. Ecol. Prog. Ser. 23, 301–306. https://doi.org/10.3354/meps023301.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179, 217–229. https://doi.org/10.1016/S0044-8486(99)00191-X.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2003. 4 - the lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition, third ed. Academic Press, San Diego, pp. 181–257. https://doi.org/10.1016/B978-012319652-1/50005-7.

Schmitz, G., Ecker, J., 2008. The opposing effects of n−3 and n−6 fatty acids. Prog. Lipid Res. 47, 147–155. https://doi.org/10.1016/j.plipres.2007.12.004.

Selleslagh, J., Amara, R., Laffargue, P., Lesourd, S., Lepage, M., Girardin, M., 2009. Fish composition and assemblage structure in three Eastern English Channel macrotidal estuaries: a comparison with other French estuaries. Estuar. Coast Shelf Sci. 81, 149–159. https://doi.org/10.1016/j.ecss.2008.10.008.

Skalli, A., Robin, J.H., 2004. Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (Dicentrarchus labrax) juveniles: growth and fatty acid composition. Aquaculture 240, 399–415. https://doi.org/10.1016/j.aquaculture.2004.06.036.

Skalli, A., Robin, J.H., Le Bayon, N., Le Delliou, H., Person-Le Ruyet, J., 2006. Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (Dicentrarchus labrax). Aquaculture 255, 223–232. https://doi.org/10.1016/j.aquaculture.2005.12.006.

Tarricone, S., Caputi Jambrenghi, A., Cagnetta, P., Ragni, M., 2022. Wild and farmed sea bass (Dicentrarchus labrax): comparison of biometry traits, chemical and fatty acid composition of fillets. Fishes 7, 45. https://doi.org/10.3390/fishes7010045.

Teichert, N., Borja, A., Chust, G., Uriarte, A., Lepage, M., 2016. Restoring fish ecological quality in estuaries: implication of interactive and cumulative effects among anthropogenic stressors. Sci. Total Environ. 542, 383–393. https://doi.org/10.1016/j.scitotenv.2015.10.068.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184. https://doi.org/10.1080/713610925.

Tocher, D.R., Bendiksen, E.Å., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280, 21–34.

Tocher, D.R., Betancor, M.B., Sprague, M., Olsen, R.E., Napier, J.A., 2019. Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: bridging the gap between supply and demand. Nutrients 11, 89. https://doi.org/10.3390/nu11010089.

Tocher, D.R., Fonseca-Madrigal, J., Dick, J.R., Ng, W.-K., Bell, J.G., Campbell, P.J., 2004. Effects of water temperature and diets containing palm oil on fatty acid desaturation and oxidation in hepatocytes and intestinal enterocytes of rainbow trout (Oncorhynchus mykiss). Comp. Biochem. Physiol. B Biochem. Mol. Biol. 137, 49–63. https://doi.org/10.1016/j.cbpc.2003.10.002.

Torrecillas, S., Robaina, L., Caballero, M.J., Montero, D., Calandra, G., Mompel, D., Karalazos, V., Kaushik, S., Izquierdo, M.S., 2017. Combined replacement of fishmeal and fish oil in European sea bass (Dicentrarchus labrax): production performance, tissue composition and liver morphology. Aquaculture 474, 101–112. https://doi.org/10.1016/j.aquaculture.2017.03.031.

Trushenski, J., Schwarz, M., Bergman, A., Rombenso, A., Delbos, B., 2012. DHA is essential, EPA appears largely expendable, in meeting the n−3 long-chain polyunsaturated fatty acid requirements of juvenile cobia Rachycentron canadum. Aquaculture 326–329, 81–89. https://doi.org/10.1016/j.aquaculture.2011.11.033.

Turchini, G.M., Francis, D.S., Senadheera, S.P.S.D., Thanuthong, T., De Silva, S.S., 2011. Fish oil replacement with different vegetable oils in Murray cod: evidence of an "omega-3 sparing effect" by other dietary fatty acids. Aquaculture 315, 250–259. https://doi.org/10.1016/j.aquaculture.2011.02.016.

Twining, C.W., Taipale, S.J., Ruess, L., Bec, A., Martin-Creuzburg, D., Kainz, M.J., 2020. Stable isotopes of fatty acids: current and future perspectives for advancing trophic ecology. Phil. Trans. Biol. Sci. 375, 20190641 https://doi.org/10.1098/rstb.2019.0641.

Vagner, M., Lacoue-Labarthe, T., Infante, J.-L.Z., Mazurais, D., Dubillot, E., Delliou, H.L., Quazuguel, P., Lefrançois, C., 2015. Depletion of essential fatty acids in the food source affects aerobic capacities of the golden grey mullet Liza aurata in a warming seawater context. PLoS One 10, e0126489. https://doi.org/10.1371/journal.pone.0126489.

Vagner, M., Pante, E., Viricel, A., Lacoue-Labarthe, T., Zambonino-Infante, J.-L., Quazuguel, P., Dubillot, E., Huet, V., Le Delliou, H., Lefrançois, C., Imbert-Auvray, N., 2019. Ocean warming combined with lower omega-3 nutritional availability impairs the cardio-respiratory function of a marine fish. J. Exp. Biol. 222, jeb187179 https://doi.org/10.1242/jeb.187179.

Vagner, M., Robin, J.H., Zambonino Infante, J.L., Person-Le Ruyet, J., 2007a. Combined effects of dietary HUFA level and temperature on sea bass (Dicentrarchus labrax) larvae development. Aquaculture 266, 179–190. https://doi.org/10.1016/j.aquaculture.2007.02.040.

Vagner, M., Robin, J.H., Zambonino-Infante, J.L., Tocher, D.R., Ruyet, J.P.-L., 2009. Ontogenic effects of early feeding of sea bass (Dicentrarchus labrax) larvae with a range of dietary n-3 highly unsaturated fatty acid levels on the functioning of polyunsaturated fatty acid desaturation pathways. Br. J. Nutr. 101, 1452–1462. https://doi.org/10.1017/S0007114508088053.

Vagner, M., Santigosa, E., 2011. Characterization and modulation of gene expression and enzymatic activity of delta-6 desaturase in teleosts: a review. Aquaculture 315, 131–143. https://doi.org/10.1016/j.aquaculture.2010.11.031. Larvi 2009.

Vagner, M., Zambonino Infante, J.L., Robin, J.H., Person-Le Ruyet, J., 2007b. Is it possible to influence European sea bass (Dicentrarchus labrax) juvenile metabolism by a nutritional conditioning during larval stage? Aquaculture, Nutrition and Feeding of Fish 267, 165–174. https://doi.org/10.1016/j.aquaculture.2007.01.031.

Vagner, M., Zambonino-Infante, J.-L., Mazurais, D., Imbert-Auvray, N., Ouillon, N., Dubillot, E., Le Delliou, H., Akbar, D., Lefrançois, C., 2014. Reduced n-3 highly unsaturated fatty acids dietary content expected with global change reduces the metabolic capacity of the golden grey mullet. Mar. Biol. 161, 2547–2562. https://doi.org/10.1007/s00227-014-2526-3.

Van Anholt, R.D., Spanings, F.A.T., Koven, W.M., Nixon, O., Bonga, S.E.W., 2004. Arachidonic acid reduces the stress response of gilthead seabream Sparus aurata L. J. Exp. Biol. 207, 3419–3430. https://doi.org/10.1242/jeb.01166.

van Meer, G., Voelker, D.R., Feigenson, G.W., 2008. Membrane lipids: where they are and how they behave. Nat. Rev. Mol. Cell Biol. 9, 112–124. https://doi.org/10.1038/nrm2330.

Vasconcelos, R.P., Henriques, S., França, S., Pasquaud, S., Cardoso, I., Laborde, M., Cabral, H.N., 2015. Global patterns and predictors of fish species richness in estuaries. J. Anim. Ecol. 84, 1331–1341. https://doi.org/10.1111/1365-2656.12372.

Viso, A.-C., Marty, J.-C., 1993. Fatty acids from 28 marine microalgae. Phytochemistry, The International Journal of Plant Biochemistry 34, 1521–1533. https://doi.org/10.1016/S0031-9422(00)90839-2.

Zheng, X., Tocher, D.R., Dickson, C.A., Bell, J.G., Teale, A.J., 2005. Highly unsaturated fatty acid synthesis in vertebrates: new insights with the cloning and characterization of a Δ6 desaturase of Atlantic salmon. Lipids 40, 13–24. https://doi.org/10.1007/s11745-005-1355-7.

Zhu, Y., Tan, Q., Zhang, L., Yao, J., Zhou, H., Hu, P., Liang, X., Liu, H., 2019. The migration of docosahexenoic acid (DHA) to the developing ovary of female zebrafish (Danio rerio). Comp. Biochem. Physiol. Mol. Integr. Physiol. 233, 97–105. https://doi.org/10.1016/j.cbpa.2019.04.005.