Dietary DHA limitation did not affect swimming and metabolic performance, but reduced growth in wild European sea bass

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Abstract

Long-chain polyunsaturated fatty acids (LC n-3 PUFA), particularly docosahexaenoic acid (DHA), are essential for cell membrane structure and function, impacting overall fish performance. These molecules are produced primarily by phytoplankton and transferred up the trophic chain; however, due to climate change, reduced DHA production by primary producers is anticipated, potentially decreasing DHA availability for fish. This study aimed to evaluate the effects of dietary DHA limitation on i) growth, swimming performance, and metabolic rates, and ii) the activation of biosynthetic pathways for DHA compensation at the molecular level, by measuring gene expression involved in DHA synthesis. We conditioned wild-caught European sea bass juveniles for five months on either a DHA-depleted or control diet. Dietary DHA limitation led to selective retention or synthesis in fish tissues (liver, brain, and white muscle), up-regulation of DHA biosynthetic pathways, and reduced growth without fully compensating for DHA deficiency in tissues. Fish fed the low DHA diet showed increased biosynthetic activity, suggesting this pathway may be energetically costly, as high tissue DHA levels correlated with reduced growth. Alternatively, the lower tissue DHA levels in these fish might directly cause slower growth. However, metabolic rates and swimming performance were not affected by dietary DHA levels. Significant inter-individual variability was observed across all variables, highlighting underlying trade-offs in coping with DHA limitation. This work provides insight into the physiological consequences of dietary DHA reduction due to global change and the molecular mechanisms fish employ to mitigate its effects.

Keywords: Critical swimming speed; metabolic rate; LC n-3 PUFA; phenotypic plasticity; *fads2* gene expression; Specific Growth Rate; dietary DHA

Author contribution

MP: Conceptualization, Investigation, Methodology, Data curation, Writing – original draft, – review & editing.

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1. Introduction

Long chain polyunsaturated fatty acids (LC-PUFA), particularly omega-3 (LC n-3 PUFA) are essential components of cell membranes in most organisms including fish (Sargent et al., 2003). Among them, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) are major FA found in membrane phospholipids (Tocher, 2003). Consequently, LC n-3 PUFA are crucial for cell function and tissue integrity, influencing the overall biological performance of the organism (Calder, 2012; Sargent et al., 2003). In fish, LC n-3 PUFA play a role in various biological processes including growth, locomotion, immunity, stress response or reproduction (Tocher, 2003).

In natural environment, marine phytoplankton are the primary producers of LC n-3 PUFA, which are then transferred to higher trophic levels (Gladyshev et al., 2013; Parrish, 2013). The availability of these FA at the base of the food web is crucial for organisms like marine fish, which have a limited ability to synthesize them de novo due to a lack of key enzymes (desaturases and elongases) involved in LC n-3 PUFA biosynthesis (Sargent et al., 2003; Tocher, 2003). Environmental changes such as ocean acidification, rising temperatures, and hypoxia have been shown to alter marine organisms' physiology, including phytoplankton, and shift community structures (Galloway and Winder, 2015; Hixson and Arts, 2016; Leu et al., 2006). These shifts may favour microalgae species less efficient in LC n-3 PUFA production while reducing the abundance of those that are more productive, leading to a decrease in LC n-3 PUFA availability (Colombo et al., 2020; Guschina and Harwood, 2006; Hixson and Arts, 2016; Holm et al., 2022).

The effects of reduced dietary LC n-3 PUFA on fish performance have been extensively studied in aquaculture, particularly in relation to growth, survival, and tissue nutritional quality (Tocher, 2015, 2010). However, few studies have explored the impact of reduced dietary LC n-3 PUFA on ecologically relevant parameters like metabolism and swimming performance (Lund et al., 2014; Vagner et al., 2015, 2014).

For instance, adult Atlantic salmon (*Salmo salar*) fed a diet rich in LC n-3 PUFA for 112 days exhibited reduced maximum aerobic swimming speed (U_{crit}) compared to fish fed on a canola oil-enriched diet lower in LC n-3 PUFA (McKenzie et al., 1998). However, other authors found that post-juvenile Atlantic salmon fed high LC n-3 PUFA levels for 31 weeks had higher U_{crit} than those fed a poultry fat-enriched diet, lower in LC n-3 PUFA (Wagner et al., 2004). Similarly, Arctic charr (*Salvelinus alpinus*) fed a high LC n-3 PUFA diet had greater U_{crit} values than those fed rapeseed and palm oil-enriched diets, which were lower in LC n-3 PUFA (Pettersson et al., 2010). In golden grey mullet (*Chelon auratus*) juveniles, dietary LC n-3 PUFA content did not affect U_{crit}, but it did increase oxygen consumption during swimming and impaired recovery capacities (Vagner et al., 2014).

However, these studies examined the effects of a combined dietary limitation of EPA and DHA. It was reported that the reduction in DHA production by microalgae is expected to be greater than for other LC n-3 PUFA (Hixson and Arts, 2016) potentially decreasing DHA availability for

higher trophic levels, including fish (Galloway and Winder, 2015; Hixson and Arts, 2016; Pethybridge et al., 2015; Poloczanska et al., 2016). Some studies have shown that DHA alone (without EPA) can meet the LC n-3 PUFA requirements for growth and survival in juvenile cobia (*Rachycentron canadum*) (Trushenski et al., 2012). DHA has been extensively studied for its benefits on vertebrate cardio-vascular function, brain development and cognition (Lauritzen et al., 2001; Sargent et al., 1999; Tocher, 2003). In teleost fish, DHA is involved in muscle fiber development by influencing mitochondrial quality control as well as improving mitochondrial function (Bian et al., 2024; Ji et al., 2024a, 2024b). Additionally, DHA may improve carbohydrate utilization, which is vital for maintaining growth and energy efficiency (Li et al., 2023). Understanding the effects of sole dietary DHA limitation on the physiology of organisms with limited ability to synthesize it is therefore critical.

The European sea bass is an active and opportunistic predator foraging mostly small fish and crustaceans, and subject to predation by bigger fish or avian predators at juvenile stage (Pickett and Pawson, 1994). Given its active nature, swimming performance may serve as a proxy for individual fitness in natural environments (Claireaux et al., 2006). To date, only one study has explored the relationship between dietary fatty acids and metabolic or swimming performance in European sea bass (Chatelier et al., 2006). This study found that a dietary limitation in LC n-3 PUFA reduced growth rates, while higher LC n-3 PUFA levels decreased metabolic rates; however, these results were based on domesticated fish, which may have genotypic differences affecting phenotypic traits (e.g., metabolism or swimming performance) compared to their wild counterparts (Albert et al., 2012; Beamish, 1978).

In this context, the aim of this study was to experimentally assess the effects of a dietary DHA limitation on (i) growth, swimming performance, and associated metabolic rates in wild European sea bass, using the critical swimming speed (U_{crit}) step protocol (Brett, 1964), and (ii) the activation of biosynthetic pathways at the molecular level to compensate for DHA limitation by measuring the expression of genes involved in fatty acid biosynthesis. We hypothesized that a five-months dietary DHA limitation will stimulate the biosynthetic pathways at the molecular level (Geay et al., 2010; Vagner et al., 2007), inducing a metabolic cost, that would consequently alter growth and metabolic performances. This study will enhance our understanding of the underexplored consequences of global change on an ecologically relevant species.

2. Material and Methods

2.1. Ethical Statement

All of the experiments were performed with respect of the regulations of the Animal Care Committee of France (ACCF). All experiments were conducted at the Ifremer facilities (Plouzané, France, Agreement number: B29-212-05) and approved by the ethical committee (DAP30512_ 2021031811505740). All precautions were taken to minimize fish suffering.

2.2. Fish Maintenance

2.2.1. Fish collection

About 150 juvenile European sea bass were captured in the Seine estuary during the NOURDEM survey in August 2021 (Le Goff et al., 2022). Fish were then immediately transported to Ifremer rearing facilities (Plouzané, France) into an oxygenated (> 120 % of air saturation) 400 L tank containing the water of the environment in which they were caught.

2.2.2. Rearing

Fish were acclimated in quarantine during 66 days in a single 2000 L tank with open circuit and natural temperature that followed the natural variation of the bay of Brest. During this period, they were fed a mix (from 25 to 100%) of frozen seafood and commercial pellets (NeoStart, Le Gouessant, Lamballe, France) in order to acclimate them to pellets. After 30 days, fish were then fed exclusively on commercial pellets for 36 days. After these 66 days of quarantine, fish (n=101; 18.9 ± 0.2 cm, 73.6 ± 2.4 g) were transferred to six experimental 400 L tanks (n=16-17 individuals per tank, total n = 101) in open circuit, exposed to a 12h light / 12h dark photoperiod cycle, and natural temperature cycle (mean = 14.5°C ± 0.14, min-max = 10.3°C-18.7°C). Before being transferred to their tanks, fish were anesthetized using MS-222 (tricaine methanesulfonate, 0.1 g L^{-1}), individually weighed, measured and individually identified with pit-tags (M120; biolog-id, Réseaumatique, Bernay, France). Fish were then progressively (from 25 to 100% of experimental diet in 2 weeks) fed with two experimental iso-proteic and iso-lipidic diets, differing in their FA content (Table 1). These diets were formulated according to the needs established for farmed European sea bass juveniles (Skalli and Robin, 2004). Both diets were manufactured at INRAE (Donzacq, France). A control and a low DHA diet were formulated using fish oil and microalgae oil (*Nannochloropsis* spp.), respectively. The two

conditions (hereafter called Control and Depleted, respectively) were reared in triplicate (n= 51 fish for the Control condition and n= 50 for the Depleted condition). Fish were fed this diet once a day (0.6-2 % of total tank biomass, depending on water temperature) for a minimum of 163 days. The oxygen saturation in the tanks was daily controlled and was never below 90%. Every month, the diet quantity was adjusted by monitoring fish growth. For that, 24h-starved fish were anesthetized with MS-222 (0.1 $g.L^{-1}$) and then weighed (± 0.1 g) and measured (± 0.1 cm).

Table 1: Formulation and fatty acid composition (% of total lipids) of the two experimental diets (Control and Depleted). ARA: Arachidonic acid; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; FA: Fatty acids; LC PUFA: Long chain polyunsaturated fatty acids; MUFA; Monounsaturated fatty acids; PUFA: Polyunsaturated Fatty acids; SFA: Saturated Fatty acids. See in the next section of the material and methods section for fatty acid analysis protocol.

Ingredients (g $100g^{-1}$)	**Control diet**	**Depleted diet**
Fish Meal	20	20
Wheat gluten	50	50
Canola oil	3	5
Fish oil	7	0
Nannochloropsis spp. Oil	0	5
Starch	6	6
Vitamins	4	4
Minerals	2	2
Cellulose	5	5
Betaine	3	3
Proximal composition (g $100g^{-1}$ ± SD)		
Proteins	59.2 ± 1.8	59.5 ± 1.8
Lipids	13.8 ± 1.0	13.1 ± 1.0
Nitrogen	9.5 ± 0.3	9.5 ± 0.3
FA composition (% of total FA ± SD)		
14:0	4.9 ± 0.5	2.2 ± 0.5
15:0	0.3 ± 0.5	0.2 ± 0.5
16:0	15.1 ± 1.2	12.8 ± 1.0
17:0	0.1 ± 0.5	0.1 ± 0.5
18:0	3.2 ± 0.5	1.2 ± 0.5
20:0	0.4 ± 0.5	0.4 ± 0.5
22:0	0.2 ± 0.5	0.2 ± 0.5
∑ SFA	24.8 ± 1.0	17.9 ± 1.5
16:1n-7	4.8 ± 0.5	9.4 ± 0.8
16:1n-9	0.1 ± 0.5	0.5 ± 0.5
18:1n-7	2.8 ± 0.5	2.0 ± 0.5
18:1n-9	22.7 ± 1.8	30.0 ± 2.4
20:1n-9	1.5 ± 0.5	0.6 ± 0.5
∑ MUFA	35.7 ± 2.3	43.8 ± 2.6
18:2n-6	12.6 ± 1.0	17.0 ± 1.4

18:3n-3	2.9 ± 0.5	5.1 ± 0.5
20:4n-6	0.8 ± 0.5	1.5 ± 0.5
20:5n-3	10.7 ± 0.9	13.2 ± 1.1
22:5n-3	1.5 ± 0.5	1.2 ± 0.5
22:6n-3	5.3 ± 0.5	0.5 ± 0.5
∑ PUFA	39.6 ± 2.5	39.5 ± 2.4
∑ LC PUFA	18.6 ± 1.2	15.7 ± 1.0
∑ n-3	22.7 ± 0.5	19.9 ± 1.0
∑ n-6	14.0 ± 1.1	19.3 ± 1.2
EPA/DHA	2.0 ± 0.1	26.4 ± 0.5
ARA/EPA	0.07 ± 0.0	0.11 ± 0.0

Analyzed by UpScience
(St Nolff, France)

Fish meal: LT70 Sopropêche; Wheat gluten: Roquette; Canola oil: Oléandes; FishOil: Polaris Omegavie®; *Nannochloropsis* spp oil: Polaris; Starch: Roquette; Vitamin premix (IU or mg kg^{-1} diet): DL-a tocopherol acetate 60 IU, sodium menadione bisulphate 5 mg, retinyl acetate 15,000 IU, DL-cholecalciferol 3000 IU, thiamin 15 mg, riboflavin 30 mg, pyridoxine 15 mg, B12 0.05 mg, nicotinic acid 175 mg, folic acid 500 mg, inositol 1000 mg, biotin 2.5 mg, calcium pantothenate 50 mg, choline chloride 2000 mg (UPAE, INRAE); Mineral premix (g or mg kg^{-1} diet): calcium carbonate (40% Ca) 2.15 g, magnesium oxide (60% Mg) 1.24 g, ferric citrate 0.2 g, potassium iodide (75% I) 0.4 mg, zinc sulfate (36% Zn) 0.4 g, copper sulfate (25% Cu) 0.3 g, manganese sulfate (33% Mn) 0.3 g, dibasic calcium phosphate (20% Ca, 18% P) 5 g, cobalt sulfate 2 mg, sodium selenite (30% Se) 3 mg, KCl 0.9 g, NaCl 0.4 g (UPAE, INRAE).

2.3. Swimming challenge

2.3.1. Experimental setup

Swimming and metabolic performances were acquired on 18 fish per experimental condition, using two swim tunnel respirometers in parallel (Loligo Systems, Tjele, Denmark) filled with the same water as those in the fish tanks. For each tunnel, the respirometer (10L) was composed of a swim chamber (40Lx10Hx10W cm) and a hydraulic system that created a laminar flow thanks to a honeycomb structure placed upstream of the swim chamber. An external motor generated the flow with a propeller and the experimenter controlled the speed. Both motors were calibrated using a current meter before the experiments. In both tunnels, a flush pump was used to maintain the oxygen level above 85% in the swim chamber. Black shelter on the side of the swim chamber and mild lightning was used to avoid any disturbance of the animal during the experiment. Experiments were continuously monitored using webcams (Logitech C920s PRO) placed above the swim chamber that were displayed live on a computer. Oxygen levels were measured using oxygen probes (FireSting O_2, PyroScience,

Germany) calibrated before each experiment and placed inside the swim chamber. The probes monitored oxygen level and temperature every second and data were acquired using the software PyroScience Workbench (V1.3.9.2140).

2.3.2. Swimming protocol

Before each experiment, two fish (one per experimental condition) were electronically identified, isolated from their rearing tanks to be fasted for a minimum of 48h (Claireaux et al., 2006; Dupont-Prinet et al., 2010). Following this fasting period, i.e. the day before the swimming test, after 4:00 pm, fish were anesthetized with MS-222, measured in a bucket filled with seawater to further adjust the current velocity in body length per second (BL s^{-1}). Fish were then transferred from the acclimation tank to the swim chamber of the swim tunnel in a plastic bag filled with seawater (without exposing the gills to the air) (McKenzie et al., 2007). After a few minutes (~10 min) recovery of the fish from the anaesthesia, fish were trained in the swim chamber for 10 min by progressively increasing the velocity of the current. After training, the fish remained overnight in the swim tunnel at a low velocity (0.5 BL s^{-1}). During all night, oxygen consumption and temperature were recorded, and the oxygen level in the swim chambers was maintained thanks to the flushing pump with cycles of 15 min of measurement and 5 min of flushing. The following morning, a step protocol experiment (Brett, 1964) was conducted to measure swimming performance. Swimming performance was assessed by measuring the critical swimming speed (U_{crit}; the maximum velocity a fish can sustain aerobically; Brett, 1964) and the associated oxygen consumption. With intervals of 20 min, the current velocity was increased for 5 min following this protocol: increments of 1.5 BL s^{-1} from 0.5 to 3.5 BL s^{-1}, then increments of 0.75 BL s^{-1} until fish exhaustion.

During each swimming step of the protocol (20 min), the flushing pump was turned off and decreasing oxygen level corresponding to fish oxygen consumption was measured. During the speed-up steps (5 min), the flushing pump was turned on, allowing the restoration of oxygen levels that were never under 75%. Fish were considered exhausted when they failed to swim off the grid for 15 seconds after light stimulation. When the exhaustion was reached, the velocity of the current was immediately reduced to the recovery speed (0.5 BL s^{-1}). The fish then entered a recovery period during which the oxygen consumption was measured, as previously described (i.e. 20 min measurement, 5 min flush) for 1 hour (= 3 measurements),

to assess the fish recovery capacity in terms of excess post-oxygen consumption (EPOC). EPOC quantifies the energy and time required to restore tissue and cell storage of oxygen, metabolite balance, such as lactate and glycogen, and other metabolic functions such as ionic and osmotic balance (Lee et al., 2003).

After the recovery period, the fish was taken out of the swim chamber with a net, and immediately euthanized with MS-222 (0.4 g L^{-1}). Its width, height, weight and length were measured, and white muscle (sampled on the left side, just anterior to the head), liver (without gallbladder), and brain were immediately sampled and flash frozen in liquid nitrogen for further fatty acid and gene expression analyses. Before and after each experiment, the background microbial respiration was measured for 1h in the swim chamber without fish. At the end of each experiment, the swim tunnel was fully cleaned with bleach tabs and rinsed three times with fresh water. The tunnels were then rinsed with and filled again with UV-treated sea water. During the swimming challenge, three fish (2 in the Control condition and 1 in the Depleted condition), apparently healthy, died overnight, during the recovery period before the swimming test. At the end of the experiment, 16 fish remained in the Control condition and 17 fish remained in the Depleted condition.

2.4. Calculation of metabolic and swimming performance variables

2.4.1. Growth performance

For each individual, the Specific Growth Rate (SGR; % per day; Lugert et al., 2016) was calculated over the 5 months experimental period using the following equation (1):

$$SGR = 100 \times \left(\frac{log(W_{final}) - log(W_{initial})}{D}\right) (1)$$

Where W_{final} is the weight at the end of the experiment, $W_{initial}$ is the weight at the beginning of the experiment and D is the number of days between the disposal into experimental condition and the end of the experiment (162-192 days).

Fulton condition factor (CF, arbitrary unit) (Fulton, 1904) was also calculated over the 5 months of conditioning using the following equation (2):

$$CF = 100 \times \left(\frac{W}{TL^3}\right) (2)$$

Where *W* is the weight (g) and *TL* is the total length (cm) of the fish.

2.4.2. Critical swimming speed

U_{crit} (BL s^{-1}) was calculated based on the following equation (3) (Brett, 1964):

$$U_{crit} = U_{max} + \frac{t_1}{t} \times U_1 \quad (3)$$

Where U_{max} (BL s^{-1}) is the highest velocity reached by the individual during a whole swimming step (i.e. 20 min), t_1 (min) is the time the individual spent at the exhaustion velocity, *t* (min) is the time of a complete swimming phase (i.e. 20 min), and U_1 (BL s^{-1}) is the increment of velocity before exhaustion of the fish (i.e. 1.5 or 0.75 BL s^{-1}).

Correction for solid blocking effect was made for all fish (Bell and Terhune, 1970), and the maximum cross section area of the fish ranged from 7 to 14% of the swim section area for length ranging from 17.6 to 23.4 cm.

2.4.3. Oxygen consumption

The MO_2 (mg O_2 kg^{-1} h^{-1}) was calculated based on the equation (4) (Lefrançois and Claireaux, 2003).

$$M_{O_2\,meas} = \left[\left(\frac{(\Delta[O_2])}{\Delta t} - \frac{\Delta O_2}{\Delta t}_{bact}\right)\right] \times \left(\frac{v}{m}\right) \quad (4)$$

Where $\Delta[O_2]$ (mg O_2 L^{-1}) is the decrease in oxygen concentration relative to oxygen consumption measured in the swim chamber over time Δt (hours), *v* is the volume of the swim tunnel (10 L) minus the volume of the fish, and m is the fish weight (kg). $\Delta[O_2]/\Delta t$ is obtained by plotting $[O_2]$ versus time to adjust a linear regression (Graphical Analysis 3.4, Beaverton, OR, USA). Only slopes with a coefficient of regression (R^2) above 0.95 were considered. The slope corresponding to the microbial background respiration was calculated before and after each experiment, and the $\Delta[O_2]/\Delta t$ *bact,* which is the mean of these two measurements, was subtracted from the MO_2 measured with fish.

As the weight of an animal influences its respiratory metabolism, MO_2 was standardized with a standard fish mass of 100 g (MO_2) (Lefrançois et al., 2001; Schurmann and Steffensen, 1997), with the following equation (5):

$$M_{O_2} = M_{O_{2\,meas}} \times \left(\frac{m}{m_{corrected}}\right)^{1-A} (5)$$

Where MO_{2meas} is the calculated oxygen consumption obtained with the equation (5), m is the fish weight (kg), and $m_{corrected}$ is the standard mass (100 g). A is the allometric exponent of the relationship between metabolic rate and fish mass. We used a value of 0.8 as it has been previously used for *Dicentrarchus labrax* (Claireaux et al., 2006), and commonly used in teleost species (Kunz et al., 2018).

2.4.4. Metabolic rates

Standard Metabolic rate (SMR; mg O_2 kg^{-1} h^{-1}) was extrapolated at the intercept (6) (i.e. MO_2 when U = 0 BL s^{-1}; Brett, 1964):

$$M_{O_2} = SMRe^{bU} (6)$$

Where SMR is the intercept, b is a constant and U is the swimming speed (BL s^{-1}).

Maximal Metabolic Rate (MMR; mg O_2 kg^{-1} h^{-1}) was estimated by taking the highest value of MO_2 during the swimming challenge (Norin and Clark, 2016).

Aerobic scope (AS; mg O_2 kg^{-1} h^{-1}) was then calculated with the following equation (7) (Lefrançois and Claireaux, 2003):

$$AS = MMR - SMR (7)$$

EPOC (mg O_2 kg^{-1}) was calculated by (1) subtracting the baseline value of MO_2 (i.e. the value of MO_2 measured just before starting the experiment) from the three MO_2 values calculated for the post exercise phase, then (2) integrating the area bounded between this new MO_2 values and time using Graphical Analysis vs 3.4 (Beaverton, OR, USA) (Lee et al., 2003). EPOC is the energy and time needed to return metabolic processes to their resting state, following intense exercise (Lee et al., 2003).

The net cost of transport at maximum exercise (NCOT; mg O_2 kg^{-1} m^{-1}) was calculated as in (Clark et al., 2011) following equation (8):

$$NCOT = \frac{(MMR - SMR)}{U_{crit}} (8)$$

Where U_{crit} is expressed in m min^{-1}, MMR and SMR in mg O_2 kg^{-1} min^{-1}.

2.5. Fatty acid analysis

The fatty acid composition of liver, muscle and brain of all individuals tested in the swim tunnel ($n_{Control}$=15; $n_{Depleted}$=16) was measured. For each tissue, total lipids were extracted by grounding flash frozen tissues (whole liver, whole brain, and about 100 mg of muscle) into a homogeneous powder in liquid nitrogen, and by diving ~50 g of each tissue into a 6 mL mixture of $CHCl_3$/MeOH (2:1, v/v; Folch, 1957). The rest of the grounded liver was preserved at -80°C for further gene expression measurement (see 2.6).

2.5.1. Lipid class separation

For each total lipid sample, extracted lipids were separated into neutral lipid (NL, corresponding to the reserve lipids) and polar lipid (PL, corresponding to the membrane lipids) fractions following the method described by Le Grand et al., (2014), and using an aliquot (⅙) of the total lipid extract. Briefly, the lipid extract aliquot was evaporated to dryness under nitrogen, recovered with three washings of 0.5 mL of chloroform/methanol (98:2, v/v), and deposited at the top of a silica gel column (40 mm × 4 mm, silica gel 60 A 63–200 µm rehydrated with 6% H_2O (70–230 mesh)). NL were eluted with 10 mL of chloroform/methanol (98:2, v/v) and collected in a glass vial containing 2.3 µg of internal standard (tricosanoic acid, C23:0). PL were then eluted with 20 mL of MeOH and collected in the same way as NL. Samples from both fractions were evaporated to dryness using a rotating evaporator (Genevac).

2.5.2. Transesterification of neutral and polar lipid fractions

NL and PL fractions were processed to obtain Fatty Acid Methyl Esters (FAME), following a protocol modified from Mathieu-Resuge et al., (2019). 1 mL of KOH/MeOH (5.6:94.4; v/v) was added. Samples were then heated at 80°C during 30 min. After cooling, 800 µL of H_2SO_4/MeOH (3.4%, v/v) was added and heated at 100°C for 10 min in a dry bath. After cooling, 800 µL of hexane and 1.5 mL of hexane-saturated distilled water were added to the mixture. After a homogenization and centrifugation at 738 g during 1 min at 20 °C, a removal of the aqueous phase was realized, the hexane phase, containing FAME, was washed two more times with hexane-saturated distilled water.

2.5.3. FAME analysis

FAME were analyzed in a Varian CP8400 gas chromatograph (GC; Varian Inc., CA, USA) coupled with a flame-ionization detector (FID) as described by Mathieu-Resuge et al., (2019). FAME were injected in split less-mode in parallel on two different columns (DBWAX 30 m × 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 time using those of commercial standards (Supelco 37 Component FAME Mix, PUFA No.1 and No.3, and Bacterial Acid Methyl Ester Mix, Sigma, MO, USA). 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. We calculated the fish tissue (percentage in PL)/diet ratio (percentage in total lipids) for DHA and EPA.

2.6. Gene expression analysis

Total RNA were extracted from the frozen liver powder (n=36) 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). A 4150 TapeStation System (Agilent Technologies Inc, Santa Clara, CA, USA) was used to evaluate the RNA integrity (RIN) and only samples having a RIN ≥ 8 were used (Control n= 16; Depleted n = 14). For each sample, 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 levels of relative expression of five genes of interest were investigated by qPCR using a CFX96 Touch Real-Time PCR Detection system (Bio-Rad Laboratories Inc.). The five genes of interest were: fatty acid desaturase 2 (*fads2*), lipoprotein lipase (*lpl*), Stearoyl-CoA desaturase 1b (*scd1b*), fatty acid elongase 6 (*elovl6*) and fatty acid synthase (*fas;* Table S1). These genes were selected because they play a role in LC-PUFA or lipoprotein metabolism (Rimoldi et al., 2016). The expression of these transcripts of interest was expressed relative to that of three housekeeping genes (elongation factor 1-alpha *ef1*; beta actin *actin,* and ribosomal protein L13a *l13a*; Table S1) that met the stability requirements of the CFX Maestro software (average M value=0.68). Primers were designed from mRNA sequences available for *D. labrax* on GenBank to obtain PCR products ranging from 150 to 200 bp using Primer 3 software (Table

S1). The amplicon specificity was checked looking at the unique melting curve. Relative transcript levels were normalized to these reference genes using the ΔΔCt method (Livak and Schmittgen, 2001), with the control samples automatically selected by the software.

2.7. Statistical analysis

All analyses were performed using RStudio (v.4.2.1). The effect of dietary treatment on growth performance, metabolic variables (SMR, MMR, AS, EPOC and NCOT), FA proportions, gene expression were tested with linear mixed models (*lme4* package, *lmer* function). If necessary (i.e non-normal residuals), data were transformed to log (gene expression data). In all linear models, we used dietary treatment (i.e. two levels: Depleted and Control) as a fixed factor, and the tank number as a random factor. The significance of models was tested using type II ANOVA after ensuring that the assumptions were met (package *performance*, *check_model* function). Finally, the correlations between DHA proportions and growth performance were tested using Spearman's correlation. Significance was accepted at $p<0.05$.

3. Results

3.1. Growth performance

The results concerning growth performance are summarized in Table 2. The SGR calculated over the 162-192 days of experiment was significantly two times lower in the Depleted groups compared to the Control groups (Table 2). Three individuals from the Depleted condition had negative SGR but none in the Control condition. However, neither TL nor weight, all calculated at the end of the experiment, were significantly affected by the dietary treatment ($p>0.05$). Fulton's K showed a non-significant trend ($p=0.05$). In the Depleted group, the SGR was negatively correlated with the DHA proportion in muscle, but not with those in liver or brain (Figure 1).

Table 2: Growth performance and condition factor of European seabass juveniles fed the Control diet (n=15) or the Depleted diet (n=16). SGR: Specific Growth Rate (% per day) calculated over the experimental period (i.e. 162-192 days); CF: Condition Factor (Fulton's K, Arbitrary Unit AU), TL: Total Length (cm) and final weight (g) calculated at the end of the experimental period (i.e. at 162-192 days). Data are presented as mean ± Standard Error of the Mean (SEM). Statistical difference is expressed by the p-value obtained from an Anova on the mixed linear models.

	Control group	Depleted group	p-value	Df	F value
SGR (% per day)	0.1 ± 0.0	0.05 ± 0.0	0.04	1	4.6

Final CF (AU)	1.2 ± 0.0	1.1 ± 0.0	0.05	1	4.1
Final TL (cm)	21.1 ± 0.3	20.8 ± 0.4	0.59	1	0.3
Final Weight (g)	114.9 ± 5.6	105.9 ± 6.9	0.32	1	1.0

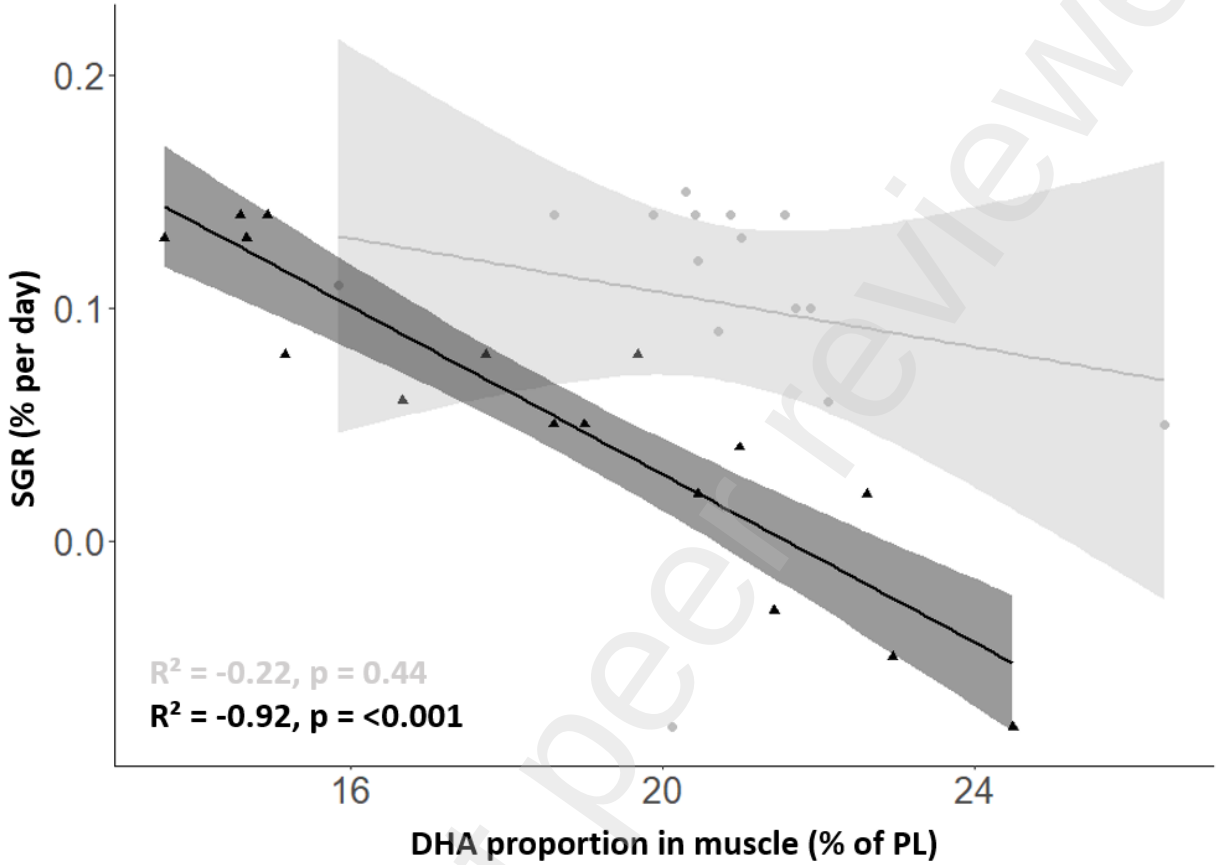


Figure 1: Spearman correlation between Specific Growth Rate (SGR) and proportion of DHA in polar lipids (PL) of muscle. The grey regression line represents the Control condition (n=15) and the black regression line represents the Depleted condition (n=16).

3.2. Fatty acid composition

3.2.1. Fatty acid composition in PL

The fatty acid composition of fish is presented in Tables 3-6.

In PL of all tissues (Table 3, 4, 5), the main FA families (SFA, MUFA, PUFA) were present in similar proportions in both Control and Depleted dietary groups ($p>0.05$). Fewer differences in FA composition were found in the brain between the two groups (only two FA, including 24:1n-9 and DHA) compared to the liver and muscle (six FA).

The DHA proportions in PL were lower in all organs of the Depleted fish compared to the Control fish. This difference in DHA was the greatest in the liver, where DHA was 28% lower in the Depleted group compared to the Control group, while it was 15% and 11% lower in the

muscle and brain of Depleted fish compared to Control fish, respectively. The EPA proportions in PL were higher in the Depleted group compared to the Control group in the liver and in the muscle. This resulted in a twofold higher EPA/DHA ratio in the liver of the Depleted group compared to the Control group (Table 3).

The total n-3 proportions in PL did not follow the same trend as DHA, and was significantly lower only in the liver of the Depleted group, but not in the muscle nor in the brain.

The arachidonic acid (ARA, 20:4n-6) proportions were higher in the Depleted group in both the muscle and the liver, but not in the brain. However, 18:2n-6 proportions were similar between groups despite the dietary differences (Table 3, Table 4, Table 5).

3.2.1. Fatty acid composition in NL

Concerning NL (Table 6), the fatty acid profile in muscle reflected the FA profile of the diet more than in PL, especially for 14:0, 16:1n-7 and 18:1n-9. DHA was 1.5 times higher in the Control group compared to the Depleted group.

In both dietary conditions, all tissues showed higher levels of DHA in the tissues in both NL and PL compared to the DHA contained in the diet. Although DHA was present at only 0.5% in the Depleted diet, it was present in the NL and PL of the three tissues, with higher proportions in the PL than in the NL.

The ratio of DHA and EPA between tissue and diet is shown in Figure 3. If this ratio is greater than 1, this indicates that the FA is selectively retained or synthesised in the tissue, reflecting that the dietary availability of this FA was below the physiological requirement. If this ratio is equal to one, it indicates that the supply of this FA meets the physiological requirement. For both experimental groups the tissue/diet DHA ratio was above 1 and was more than 30 times higher for the Depleted group compared to the Control group (Figure 3A). For the Depleted group, the brain had the highest ratio and liver had the lowest, but these ratios were still very high (37.9 and 32.8, respectively). The tissue/diet EPA ratio was above 1 in the liver and muscle, but was lower than one in the brain (Figure 3B).

Table 3: Fatty acid profiles of Polar Lipids (% of total FA) in the liver of European sea bass juveniles fed the Control (n=15) and the DHA Depleted (n=16) diets. PL: polar lipids. Data are presented as mean ± SEM. Statistical significance is indicated by the p-value.

PL FA	Liver	Statistics

	Control	Depleted	p-value
16:0	16.7 ± 0.5	15.7 ± 0.4	0.12
18:0	10.8 ± 0.4	11.2 ± 0.4	0.42
24:0	0.1 ± 0.0	0.2 ± 0.0	0.04
∑ SFA	28.8 ± 0.5	28.3 ± 0.2	0.45
16 :1n-7	1.7 ± 0.1	2.0 ± 0.1	0.02
16 :1n-9	0.8 ± 0.0	0.8 ± 0.0	0.33
18 :1n-7	2.5 ± 0.1	2.6 ± 0.1	0.47
18 :1n-9	16.0 ± 0.6	16.5 ± 0.4	0.27
20 :1n-9	0.7 ± 0.0	0.8 ± 0.0	0.002
24 :1n-9	0.4 ± 0.0	0.3 ± 0.0	0.70
∑ MUFA	22.5 ± 0.7	23.5 ± 0.5	0.12
18:2n-6	4.7 ± 0.1	5.1 ± 0.3	0.09
18 :3n-3	0.7 ± 0.0	0.8 ± 0.1	0.09
20 :4n-6	3.7 ± 0.1	5.4 ± 0.1	<0.001
20 :5n-3	11.2 ± 0.3	15.2 ± 0.4	0.002
22 :5n-3	1.6 ± 0.1	1.5 ± 0.1	0.08
22 :6n-3	22.8 ± 0.4	16.4 ± 0.7	0.002
∑ PUFA	48.0 ± 0.6	47.3 ± 0.5	0.38
∑ LC-PUFA	40.5 ± 0.7	39.3 ± 0.7	0.14
∑ n-6	9.9 ± 0.2	12.0 ± 0.3	<0.001
∑ n-3	37.2 ± 0.6	34.4 ± 0.6	0.03
∑ DMA	0.4 ± 0.0	0.5 ± 0.1	0.11
EPA/DHA	0.5 ± 0.0	1.0 ± 0.1	0.02

424

425 Table 4: Fatty acid profiles of Polar Lipids (% of total FA) in the muscle of European sea bass juveniles fed the
426 Control (n=15) and the DHA Depleted (n=16) diets. PL: polar lipids Data are presented as mean ± SEM. Statistical
427 significance is indicated by the p-value.

PL FA	Muscle		Statistics
	Control	Depleted	p-value
16:0	20.5 ± 0.5	21.1 ± 0.2	0.25
18:0	7.6 ± 0.2	7.2 ± 0.1	0.63
24:0	0.2 ± 0.0	0.2 ± 0.0	0.88
∑ SFA	29.4 ± 0.4	29.3 ± 0.2	0.77
16 :1n-7	1.5 ± 0.2	1.7 ± 0.1	0.37
16 :1n-9	0.4 ± 0.0	0.5 ± 0.0	0.05
18 :1n-7	2.3 ± 0.0	2.1 ± 0.1	0.006
18 :1n-9	14.4 ± 0.5	13.8 ± 0.3	0.29

20 :1n-9	0.6 ± 0.0	0.6 ± 0.0	0.17
24 :1n-9	0.1 ± 0.0	0.1 ± 0.0	0.99
∑ MUFA	19.7 ± 0.7	19.1 ± 0.3	0.41
18:2n-6	5.5 ± 0.2	5.5 ± 0.2	0.86
18 :3n-3	0.8 ± 0.0	0.9 ± 0.0	0.005
20 :4n-6	2.5 ± 0.1	3.4 ± 0.1	<0.001
20 :5n-3	13.7 ± 0.4	16.2 ± 0.5	<0.001
22 :5n-3	2.4 ± 0.0	2.0 ± 0.1	0.026
22 :6n-3	20.8 ± 0.6	18.6 ± 0.8	0.04
∑ PUFA	48.9 ± 0.7	49.7 ± 0.4	0.93
∑ LC-PUFA	41.4 ± 0.8	41.4 ± 0.7	0.98
∑ n-6	9.7 ± 0.1	10.7 ± 0.3	0.002
∑ n-3	37.0 ± 0.9	36.8 ± 0.7	0.32
∑ DMA	2.0 ± 0.1	1.9 ± 0.1	0.91
EPA/DHA	0.7 ± 0.0	0.9 ± 0.1	<0.001

428

429 Table 5: Fatty acid profiles of Polar Lipids (% of total FA) in the brain of European sea bass juveniles fed the
430 Control (n=15) and the DHA Depleted (n=16) diets. PL: polar lipids. Data are presented as mean ± SEM. Statistical
431 significance is indicated by the p-value.

PL FA	**Brain**		**Statistics**
	Control	**Depleted**	**p-value**
16:0	17.0 ± 0.2	16.5 ± 0.3	0.21
18:0	9.1 ± 0.2	8.9 ± 0.1	0.34
24:0	1.7 ± 0.0	1.8 ± 0.1	0.22
∑ SFA	27.9 ± 1.2	28.4 ± 0.4	0.76
16 :1n-7	2.4 ± 0.0	2.5 ± 0.0	0.52
16 :1n-9	1.5 ± 0.0	1.5 ± 0.0	0.19
18 :1n-7	1.5 ± 0.0	1.4 ± 0.0	0.47
18 :1n-9	19.7 ± 0.3	19.7 ± 0.2	0.95
20 :1n-9	1.3 ± 0.0	1.3 ± 0.0	0.62
24 :1n-9	4.6 ± 0.1	4.9 ± 0.2	0.37
∑ MUFA	33.1 ± 1.3	32.4 ± 0.4	0.61
18:2n-6	0.5 ± 0.0	0.5 ± 0.0	0.84
18 :3n-3	0.1 ± 0.0	0.1 ± 0.0	0.31
20 :4n-6	3.3 ± 0.0	3.5 ± 0.1	0.11
20 :5n-3	3.5 ± 0.1	3.7 ± 0.1	0.08
22 :5n-3	0.9 ± 0.0	1.0 ± 0.0	0.07
22 :6n-3	20.8 ± 0.3	19.4 ± 0.5	0.04
∑ PUFA	31.3 ± 0.3	30.7 ± 0.5	0.47

∑ LC-PUFA	29.1 ± 0.2	28.3 ± 0.5	0.13
∑ n-6	4.8 ± 0.1	5.2 ± 0.2	0.03
∑ n-3	26.0 ± 0.3	24.9 ± 0.5	0.26
∑ DMA	7.4 ± 0.2	8.3 ± 0.3	0.03
EPA/DHA	0.17 ± 0.0	0.2 ± 0.0	0.06

432

434 *Table 6: Fatty acid profiles of Neutral Lipids (% of total FA) in the muscle of European sea bass juveniles fed the*
435 *Control (n=15) and the DHA Depleted (n=16) diets. NL: neutral lipids. Data are presented as mean ± SEM.*
436 *Statistical significance is indicated by the p-value.*

NL FA	Muscle		Statistics
	Control	Depleted	p-value
14:0	2.8 ± 0.1	2.3 ± 0.1	<0.001
16:0	19.0 ± 0.2	18.8 ± 0.2	0.52
18:0	4.2 ± 0.1	3.9 ± 0.1	0.20
∑ SFA	26.8 ± 0.3	25.8 ± 0.3	0.11
16 :1n-7	5.6 ± 0.1	7.1 ± 0.2	<0.001
18 :1n-7	3.2 ± 0.0	3.0 ± 0.1	0.32
18 :1n-9	30.2 ± 0.3	32.6 ± 0.3	<0.001
20 :1n-9	1.8 ± 0.0	1.8 ± 0.1	0.43
∑ MUFA	43.2 ± 0.4	47.0 ± 0.5	<0.001
18 :2n-6	9.5 ± 0.2	9.2 ± 0.4	0.66
18 :3n-3	1.6 ± 0.0	1.8 ± 0.1	0.07
20 :4n-6	0.5 ± 0.0	0.7 ± 0.0	<0.001
20 :5n-3	6.6 ± 0.3	6.9 ± 0.3	0.58
22 :5n-3	1.2 ± 0.1	0.7 ± 0.1	<0.001
22 :6n-3	4.4 ± 0.1	2.8 ± 0.1	<0.001
∑ PUFA	28.7 ± 0.5	26.2 ± 0.5	<0.001
∑ LC-PUFA	14.3 ± 0.4	12.5 ± 0.4	0.005
∑ n-6	11.4 ± 0.2	11.0 ± 0.7	0.58
∑ n-3	13.8 ± 0.5	12.2 ± 0.4	<0.001
∑ DMA	0.5 ± 0.0	0.3 ± 0.0	<0.001
EPA/DHA	1.5 ± 0.1	2.6 ± 0.2	<0.001

437

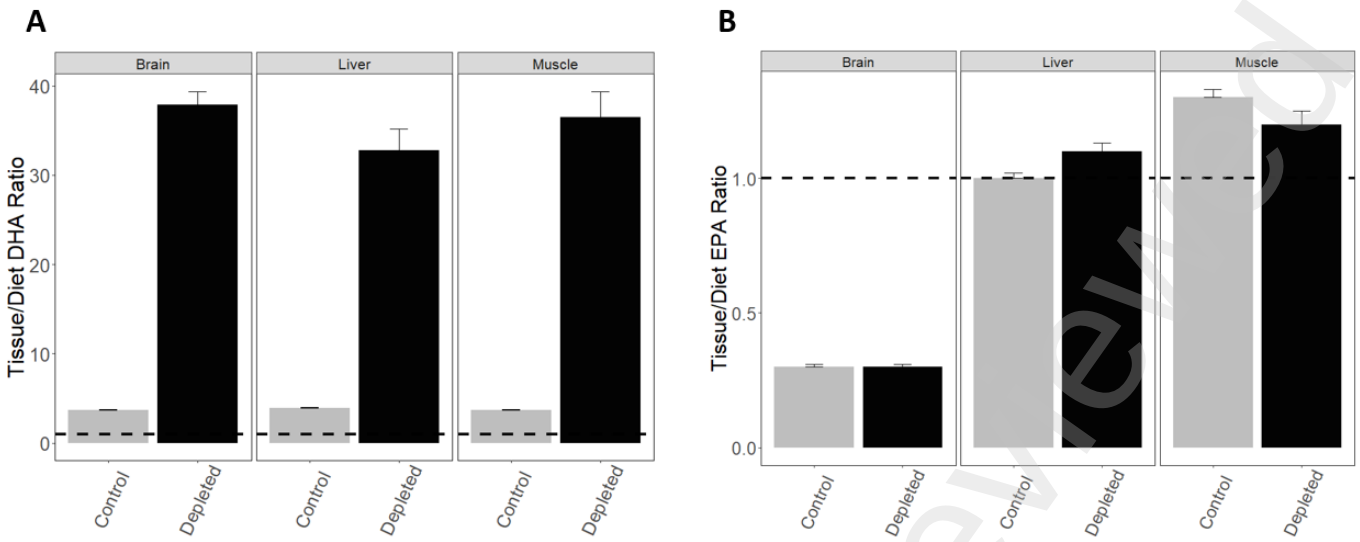
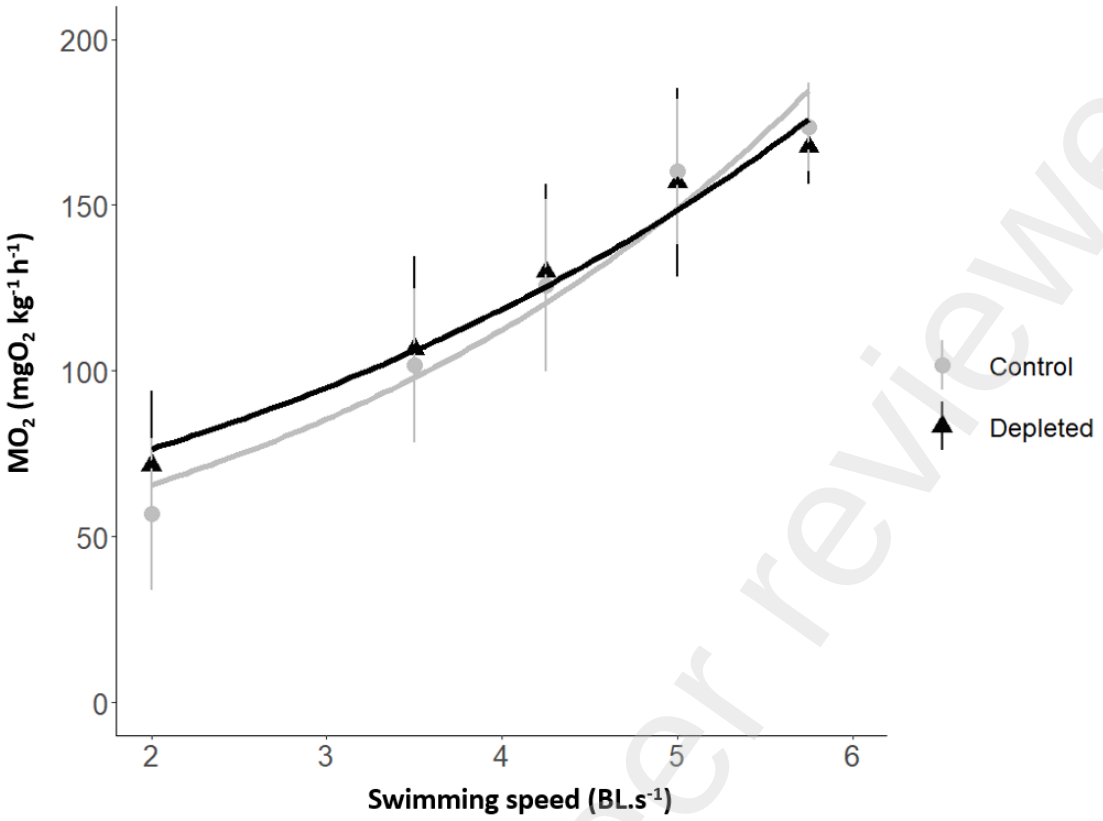


Figure 2: Ratio of FA between tissue and diet (± SEM) for DHA (A) and EPA (B) of European sea bass juveniles fed either a control or a DHA depleted diet. The grey bars represent the Control condition (n=15) and the black bars represent the Depleted condition (n=16). The horizontal dotted line represents the 1:1 ratio between the tissue and the diet. A ratio greater than 1 indicates that the FA is selectively retained or synthesised in the tissues, indicating that the dietary availability of this FA was below the physiological requirement. When this ratio is equal to one, it indicates that the supply of this FA meets the physiological requirement.

3.3. Swimming and metabolic performance

As expected, the MO_2 increased exponentially with the swimming speed for both dietary treatments (Figure 4). The metabolic variables (SMR, MMR, AS, EPOC and NCOT) and U_{crit} were not affected by the dietary treatment ($p>0.05$; results summarized in Table 7).



443

444 Figure 3: Oxygen consumption (MO_2, mg O_2 kg^{-1} h^{-1}, ± SEM) as a function of swimming speed (body length BL s^{-1})
445 for both dietary groups for European sea bass juveniles fed either a control or a DHA depleted diet. The Control
446 group is represented by circles in grey (n=14) and the Depleted group is represented by the triangles in black
447 (n=16). Both lines are exponential curves fitted on MO_2 as a function of swimming speed. (Control group curve:
448 $y=32.833e^{0.3059x}$; $r^2 = 0.99$; Depleted group curve: $y=46.094e^{0.2356}$; $r^2=0.99$).

449

450 Table 7: Metabolic and swimming performance for European sea bass juveniles fed either a Control diet or a DHA
451 Depleted diet. Data are standard metabolic rate (SMR, mg O_2 kg^{-1} h^{-1}), maximum metabolic rate (MMR, mg O_2
452 kg^{-1} h^{-1}), aerobic scope (AS, mg O_2 kg^{-1} h^{-1}), excess post-oxygen consumption (EPOC, mg O_2 kg^{-1}), critical swimming
453 speed (Ucrit, body length BL s^{-1}), and net cost of transport (mg O2 kg^{-1} m^{-1}). Data are presented as mean ± SEM,
454 n is the number of individuals statistically tested.

Dietary Condition	SMR	MMR	AS	EPOC	U_{crit}	NCOT
Control	27.1 ± 0.4 (n=14)	165.8 ± 1.8 (n=14)	138.6 ± 1.7 (n=14)	1623.5 ± 41.7 (n=14)	4.8 ± 0.1 (n=14)	2.3 ± 0.1 (n=14)
Depleted	27.0 ± 0.5 (n=16)	155.3 ± 1.6 (n=16)	128.2 ± 1.5 (n=16)	1463.4 ± 32.8 (n=16)	4.7 ± 0.1 (n=16)	2.2 ± 0.1 (n=16)

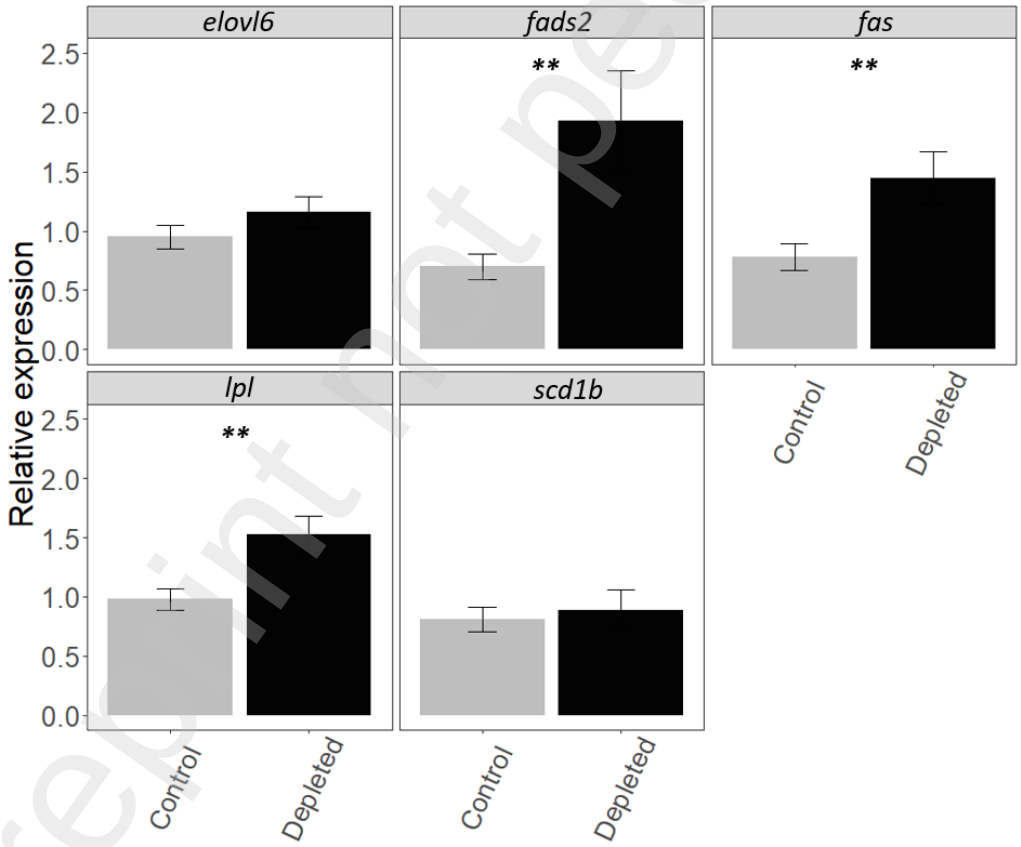
p-value	0.81	0.38	0.26	0.43	0.49	0.55
F value	0.07	0.9	1.4	0.62	0.48	0.02

455

456 3.4. Gene expression

457 The relative expression of *fads2*, *fas* and *lpl* transcripts measured in the liver were significantly
458 higher in the Depleted fish than in the Control fish ($p<0.01$, Figure 5). However, the relative
459 gene expression of *elovl6* and *scd1b* were not significantly different between the two dietary
460 groups (Figure 5).

461 Significant negative correlations were found between the hepatic DHA proportions and the
462 relative expressions of *fads2*, *fas*, *lpl* and *scd1b* for the Depleted group, but not for the Control
463 one (Figure 6).



464

465 Figure 4: Relative expression of genes (± SEM) coding for enzymes involved in lipid metabolism and measured in
466 the liver of European sea bass juveniles fed either a Control (n=16) or a DHA Depleted (n=14) diet. *elovl6* : fatty
467 acid elongase 6, *fads2*: fatty acid desaturase 2, *fas*: fatty acid synthase, *lpl*: lipoprotein lipase, *scd1b*: stearoyl-

CoA desaturase. Asterisks represent statistical significance between the two dietary conditions. Linear mixed model. ** p<0.01.

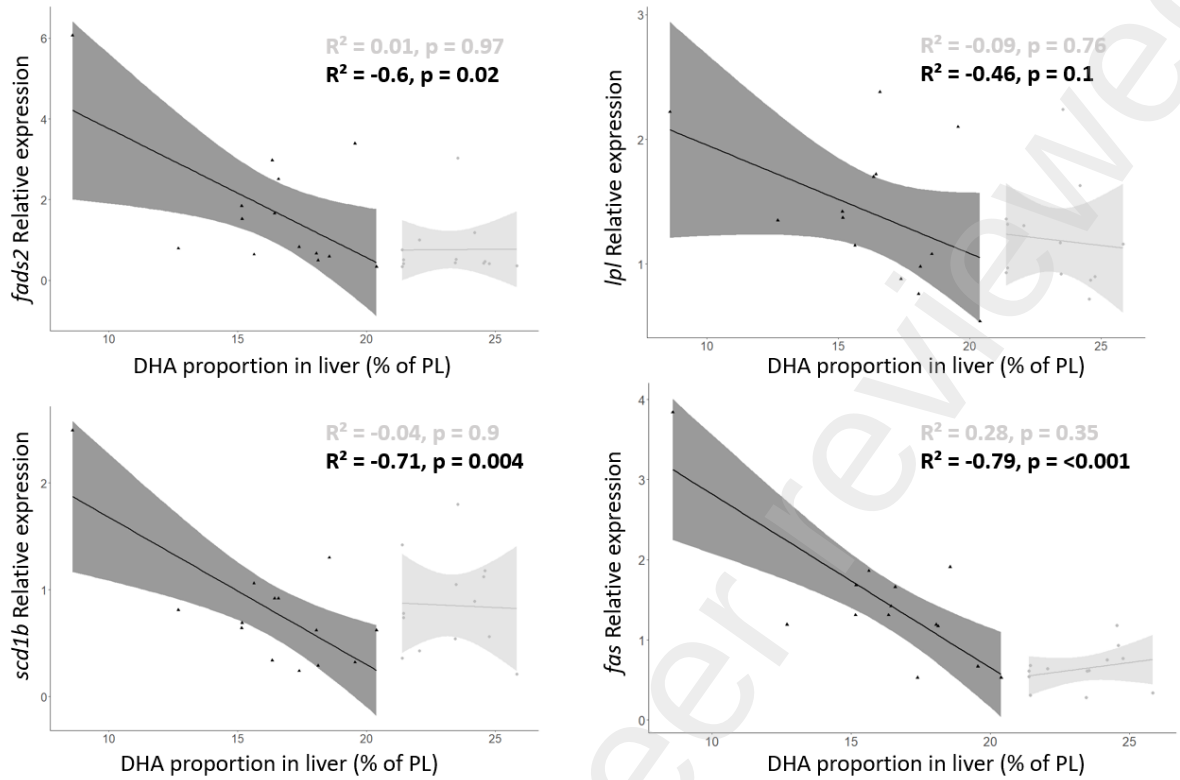


Figure 5: Spearman correlation between the DHA content in polar lipid (PL) of liver and the relative transcript expressions of *fads2* (A), *fas* (B), *lpl* (C) and *scd1b* (D). The grey regression line represents the Control condition (n=16) and the black regression line represents the Depleted condition (n=14).

4. Discussion

We investigated the effects of a long-term (5 months) dietary DHA limitation on wild-caught European sea bass juveniles, in terms of growth, FA composition in tissue, swimming performance and metabolic rates. The DHA proportions in the tissues of DHA Depleted fish were significantly reduced compared to the Control fish, although they remained high considering the extreme DHA limitation in their diet. The dietary DHA limitation negatively affected growth, but did not modulate swimming performance nor metabolic rates. We found high inter-individual variations in all measured traits, suggesting phenotypic plasticity.

4.1. Consequences of dietary DHA limitation on tissue FA proportion, gene expression and fish growth

The fatty acid composition of fish tissue reflected that of their diet, especially for LC PUFA. In particular, the dietary DHA deficiency was reflected in muscle, brain and liver.

In brain, results show that despite slightly more elevated ARA levels and the alterations in DHA, the overall LC PUFA content in PL remained relatively stable, suggesting that brain lipid homeostasis might be more resistant to dietary depletion than liver and muscle (Pilecky et al., 2021). This suggests the preservation of neural and cognitive functions of fish, which is in accordance with previous studies (Carvalho et al., 2022; Skalli et al., 2006; Vagner et al., 2024).

The calculation of the PL tissue/diet DHA ratio, that was well above one in all tissues of the depleted group, suggests that DHA requirements were not met in the fish fed the Depleted diet. When the value of this ratio is above 1, it suggests that the availability of these FA were below the physiological needs of fish and that it occurs either a selective retention in the tissue, or a synthesis of these FA. Hence, it suggests that a selective retention or an internal synthesis of DHA occurred in their tissues in order to compensate for the dietary deficiency (Závorka et al., 2021). Despite of that, and as said earlier, this was not enough to reach the same DHA levels in those tissues as in the control group. Interestingly, and to a lesser extent, the PL tissue/diet DHA ratio was also above one for the Control group, suggesting that the DHA requirements were not totally met for this group either, and that these fish also retained or synthesized DHA. The Control diet was formulated according to the needs established for European sea bass juveniles (from 14 to 26g) from aquaculture origin (Skalli and Robin, 2004). This could suggest different needs for wild fish. In addition, a notable inter-individual variability in DHA proportions within each condition group was observed. Some individuals from the Depleted condition had higher tissue DHA proportions than some individuals in the Control group, despite a tenfold lower DHA level in their diet. This inter-individual variability indicates a complex regulation of DHA in the tissue membranes that could relate on a multitude of levels such as retention or synthesis. High individual variations of LC n-3 PUFA proportions in membrane were previously observed in muscle of golden grey mullets fed high (17.5 %) and low (1.2 %) levels of EPA+DHA (Salin et al., 2021). Contrary to DHA, tissue/diet EPA ratio showed a tissue specific response. In both experimental groups, it was equal or above 1 in muscle and liver, but was below 1 (at 0.5) in the brain. This suggests that EPA supply in both diets met the fish requirements and that this FA did not need to be retained as preferentially as DHA in the targeted organs. This result could also suggest that EPA might be used to synthesize DHA in the brain (Kainz et al., 2006) or catabolized, as observed in mammals (Chen and Bazinet, 2015).

The hypothesis of DHA synthesis in Depleted fish is supported by the upregulation, in liver, of the expression of *fads2*, involved in LC n-3 PUFA metabolism, as well as by the negative correlation between gene expressions and hepatic DHA content observed in this group. Notably, *fads2* encodes the delta6 desaturase, which is the first enzyme involved in the biosynthesis of LC n-3 PUFA and is considered as a limiting step of further biosynthesis (Vagner and Santigosa, 2011). This upregulation suggests an internal synthesis of DHA in the liver to maintain the levels in the tissue. Upregulation of *fads2* has already been observed in European sea bass fed low levels of LC n-3 PUFA, but this upregulation was not correlated with FADS2 enzymatic activity (Geay et al., 2010). The lower DHA levels yet observed in the liver of Depleted fish compared to Control fish could suggest either (i) that FADS2 activity and *fads2* expression were not necessarily related, or (ii) that FADS2 activity resulting from *fads2* upregulation was not sufficient to induce compensating fatty acid synthesis in the Depleted fish. The first hypothesis could be explained by a regulation at the post-transcriptional level, involving notably microRNA, inhibiting LC-PUFA biosynthesis as it was recently shown in Rabbit fish *Signatus canaliculatus* (Xie et al., 2021). The same assumption can be applied to FAS activity, as it remained stable regardless of dietary variations (Castro et al., 2016; Viegas et al., 2022).

In addition to be associated with an upregulation of *fads2*, the DHA depleted diet was associated with a lower growth rate and induced a higher inter-individual variability in growth rate between individuals. While the ingestion rate has not been measured in this study, fish were daily observed during their feeding time, and no differences of ingestion rates, that could partly explain difference in growth rates, were visually observed between the two groups. Interestingly, in the depleted group, higher the DHA proportion in muscle, lower the growth rate (SGR). Yet, DHA has been shown to play a key role in fish growth (Watanabe and Kiron, 1994). Thus, this counterintuitive relationship between growth and DHA content would suggest an energetic cost of the physiological acclimation of individuals to a low dietary DHA content (Lazzarotto et al., 2015; Murray et al., 2014; Závorka et al., 2021). The upregulation of biosynthesis pathways set up in the depleted group to synthetize DHA trying to counteract the dietary depletion may be too costly and prevent fish to allocate energy to grow.

4.2. Consequences of dietary DHA depletion on swimming and metabolic performance

Despite the lower growth rate observed and the hypothesized higher energy expenditure in the Depleted fish to counteract the dietary deficiency, metabolic rates and maximum aerobic swimming performance were similar between the two dietary groups. This suggests that some cellular mechanisms may mask or offset the expected energetic costs. Similar MMR between fish fed either a low LC n-3 PUFA diet or a control diet has been recently reported in Atlantic salmon (Závorka et al., 2021). The authors suggested that, even though the deficiency in LC n-3 PUFA decreased mitochondrial ATP production, mitochondrial metabolism could show a resilience to low levels of LC n-3 PUFA as this did not affect the MMR of fish (Závorka et al., 2021). In addition, as mentioned earlier, our study showed that some individuals from the Depleted condition had DHA levels in the range of those measured in Control individuals. This might result from the ability of some individuals to physiologically adjust their performance at the metabolic level without obvious relationship with the activation of the DHA biosynthesis pathways. Indeed, the metabolic rate is an integrative trait depending on multiple factors (ventilation, oxygen transport, mitochondrial performance), that could have been affected without being directly visible on the measured oxygen consumption. Individuals might also use different energy sources to fuel their needs. For example, fish fed the Depleted DHA diet might have used MUFA to sustain their swimming performance, as these FA were higher in the depleted diet (Chatelier et al., 2006; McKenzie, 2001). While the high EPA level in the DHA Depleted diet did not avoid the decreased growth of Depleted fish, this might have balanced out the differences in metabolic rates. Indeed, no study investigated the effects of EPA and DHA separately but it is known that a diet rich in EPA+DHA can lower fish SMR (McKenzie, 2001). Therefore, there might be an influence of EPA (and not DHA) on the activity of cellular ionic pumps that could regulate fish metabolic rates (Paige et al., 1996; Ushio et al., 1997).

5. Conclusions

To our knowledge, this study is the first to examine the effects of a dietary limitation specifically in DHA, not combined with EPA, on the metabolic and swimming performance of wild-caught fish. DHA dietary limitation resulted in selective DHA retention or synthesis in fish tissues, the upregulation of biosynthetic pathways for DHA synthesis at the molecular level, and reduced growth rate of wild European sea bass juveniles. However, the activation of biosynthetic pathway did not compensate for the DHA deficiency. One hypothesis is that the

molecular stimulation of the biosynthetic pathway might be energetically costly for fish fed the low DHA diet, as high DHA proportions were associated with lower growth. Yet, this did not translate in differences in metabolic rates or swimming performance suggesting a strong resilience of European sea bass to dietary changes. Finally, considerable inter-individual variability was observed in all the measured variables (growth, metabolic rates, U_{crit}) suggesting a strong phenotypic plasticity. Underlying mechanisms involved at different biological levels made to counteract a dietary DHA limitation like energetic trade-offs and their implication in fish growth should be investigated at the individual level.

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851 6. Supplementary material

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853 Table S1: GENBANK accession numbers and specific primers of the sequences used for each gene.

Genes	Forward primer	Reverse primer	Accession number

fads2	CCGCCGTGACTGGGTGGAT	GCACAGGTAGCGAAGGTAGTAAGACATAGA	EU647692
lpl	GATCGTGGTGGACTG	CGTCGGGTAGTGCTGGTT	AM411614
scd1b	GCTTGTGGCATACTTCATCCCTGGACTC	GGTGGCGTTGAGCATCACGGTGTA	FN868643
fas	CTAACAACATCTGCCCAGCG	GACGGTGGCAGAATTGGATC	MF566098
elovl6	ACATCACCGTGCTGCTCTACTCCTG	CCGCCACCTGGTCCTTGTAGCA	KF857297
ef1α	CTGGAGGGCAGTGAAAAGAT	CATCAAGAGCCTCCAGCAGT	AJ866727
actin	GTGCGTGACATCAAGGAGAA	AGACGCACAATCTTGAGAGCAG	AY148350
l13α	GCTTCGAGGAAATCACCAAG	CAACCTTCCATCCCTTGAAC	DT044539

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