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Rôle de l'habitat actuel et du stade ontogénique sur la capacité du bar (*Dicentrarchus labrax*) à faire face au scénario futur de baisse de disponibilité en acides gras polyinsaturés à longue chaîne n-3 dans le réseau trophique

Mickaël Péron

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Remarks on the writing of this manuscript

This manuscript is primarily written in English. However, the doctoral school (EDSML) requires at least 10% of the manuscript to be in French. To meet this requirement, I will present non-scientific information about this thesis and my activities during its preparation in French. At the end of each chapter, a summary will be written in French. All references cited throughout the manuscript can be found in the "References" section at the end.

Avant-propos

Cette thèse fait partie du projet ADJUST porté par Marie Vagner regroupant plusieurs intervenants du LEMAR. Toutes mes expérimentations sur les poissons ont été réalisées dans l'unité PFOM (maintenant nommée PHYTNESS) de l'Ifremer (tutelle du LEMAR). J'ai réalisé les analyses d'acides gras sur la plateforme LIPIDOCEAN de l'IUEM. Durant cette thèse, j'ai été encadré par Philippe Soudant (directeur de thèse, HDR), Marie Vagner (encadrante scientifique principale), Fabienne Le Grand et David Mazurais. Mon contrat doctoral s'est déroulé du 01/10/2020 au 30/09/2023 et a été financé à 50% par la Région Bretagne et à 50% par l'école doctorale des sciences de la mer et du littoral (EDSML). Le projet ADJUST a lui été financé par plusieurs sources : Projet Emergence IsBlue (7320€), EC2CO (17 027€) et Conseil Scientifique de l'IUEM (2620€). (<https://www-iuem.univ-brest.fr/lemar/projets-scientifiques/adjust/>).

Valorisation de la thèse

Liste des publications

Publications acceptées

Peron M., Simon V., Le Grand F., Soudant P., Mazurais D., Vagner M. **Non-lethal sampling method for the analysis of white muscle fatty acid profiles in European sea bass (*Dicentrarchus labrax*)**. Accepted in *Fish physiology and biochemistry*.

Publications en révision

Peron M., Hue S., Soudant P., Le Grand F., Gonzalez R., Mazurais D., Vagner M. **Spatial and ontogenetic modulation of FA composition of juvenile European sea bass (*Dicentrarchus labrax*) from two French estuaries**. In review. in *Marine Environmental Research*.

Publications en préparation

Peron M., Soudant P., Le Grand F., Mazurais D., Simon V., Lefrancois C., Vagner M. **Effects of severe DHA depletion on the swimming and metabolic performance of a wild-caught euryhaline fish**. In prep. for *Journal of Experimental Biology*.

Peron M., Simon V., Lefrancois C., Le Grand F., Mazurais D., Soudant P., Vagner M. **Effects of a long term DHA depletion on the escape response in wild-caught juvenile European sea bass (*Dicentrarchus labrax*)**. In prep for *Journal of Experimental Biology*.

Peron M., Soudant P., Le Grand F., Mazurais D., Simon V., Vagner M. **White muscle biopsy to monitor FA composition of European sea bass submitted to a dietary conditioning**. In prep for *Fish Physiology and Biochemistry*.

Liste des communications orales en congrès

Mickaël Peron, Le Grand F., Soudant P., Mazurais D., Lefrancois C., Simon V., Vagner M. **Effects of Dietary DHA Deficiency on Metabolic and Swimming Performance of Juvenile European Seabass (*Dicentrarchus labrax*) from the wild**. RespFest. La Rochelle, France. 02-03 May 2023.

Prix de la troisième meilleure présentation.

Mickaël Peron, Le Grand F., Simon V., Quemeneur J-B., Mazurais D., Soudant P., Vagner M., **Relation between fatty acid profile, growth rate and condition factor using a non-lethal sampling method in European sea bass.** *Society for Experimental Biology. Montpellier, France. 05-08 July 2022.*

Mickaël Peron, Le Grand F., Simon V., Quemeneur J-B., Mazurais D., Soudant P., Vagner M. **Non-lethal sampling method for the analysis of fatty acids in European sea bass (*Dicentrarchus labrax*).** *International Congress on the Biology of Fish. Montpellier, France. 28 June – 01 July 2022.*

Mickaël Peron, Bideau A., Le Grand F., Mazurais D., Soudant P., Vagner M. **Spatial and ontogenetic variability of omega-3 fatty acid composition in European sea bass *Dicentrarchus labrax* from two French estuaries.** *Colloque d'Ecophysiologie Animale. Montpellier, France. 02-04 Novembre 2021.*

Mickaël Peron, Bideau A., Le Grand F., Mazurais D., Soudant P., Vagner M. **Lipid content and fatty acid composition of wild European sea bass *Dicentrarchus labrax* juveniles from two French estuaries.** *Lipids in the Ocean. Aveiro, Portugal (online). 05-08 Juillet 2021.*

Médiation scientifique

Diffusion de la « capsule vidéo » lors de la **Nuit Européenne des Chercheurs** à Océanopolis. 29 septembre 2023.

Création d'une « **capsule vidéo** » de vulgarisation de mon sujet de thèse pour les journées de l'école doctorale. Diffusion de la capsule le 16 Novembre 2022. Cette vidéo est désormais disponible sur la chaîne YouTube du LEMAR.

<https://www.youtube.com/watch?v=QjZ0VUIttVs>

Participation à la **fête de la science** pour le projet OMEGA à Brest. Présentation à un public large et novice de la science autour de la thématique des omégas-3. 08 et 09 Octobre 2022.

Encadrement, enseignement et formations

Encadrement scientifique

J'ai encadré le **stage de Master 2** à l'Université de Bretagne Occidentale, parcours Biologie des Organismes Marins (IUEM) de **Romain Gonzalvez**. Romain a participé à l'acquisition et l'analyse de résultats sur la première partie de ma thèse (chapitre 1). Son sujet de stage portait l'intitulé « Comparaison de la teneur en oméga-3 LC du muscle et du cerveau du bar Européen (*Dicentrarchus labrax*) selon son stade et son lieu de vie ». **Obtention d'une bourse IsBlue pour un stage de M2 d'un montant de 3600€.**

Enseignement

- Enseignement théorique :
 - Physiologie des grandes fonctions – Licence 3 (8 heures)
 - Ecophysiologie Animale – Licence 3 (2 heures)
- Enseignement pratique :
 - Physiologie Animale Cellulaire – Licence 2 (109 heures)
 - Ecophysiologie Animale – Licence 3 (9 heures)

Liste des formations suivies pendant la thèse

Formation	Format	Formateur	Année	Heures de formation
Introduction à la statistique avec R	MOOC	Université Paris-Saclay	2020	20h
Expérimentation Animale	Formation	ONIRIS Nantes	2021	25h
Communication orale dans un	Congrès	-	2022	20h

congrès international				
The problem-solving skills for higher education learners	Formation	University of Split	2022	30h
Aide à la rédaction scientifique en anglais	Formation	UBO	2021	15h
Comprendre les fondamentaux des réseaux professionnels	Formation	UBO	2021	4h
How to pitch?	Formation	UBO	2021	3h
Intégrité scientifique et données de la recherche	Formation	UBO	2021	2h
Training event and Entrepreneurship Skills	Formation	University of Cadiz	2023	30h

Implications dans des projets annexes

Le projet PACIFIC

Le projet PACIFIC visait à évaluer l'adaptation physiologique des acides gras (AG) du muscle et du cerveau du bar européen (*Dicentrarchus labrax*) dans des conditions expérimentales à un environnement d'eau de mer plus chaud et à un potentiel hydrogène (pH) plus faible dans un avenir proche (+5°C et -0,4 unité de pH) (contrôle, environnement plus chaud, acide, plus chaud et acide, c'est-à-dire CT, WT, AT, WAT respectivement) pendant 100 jours.

Fatty acid composition adjustment in ocean warming and acidification
experimental conditions of juvenile European sea bass (*Dicentrarchus labrax*)

Romain Gonzalvez, Mishal Cohen-Rengifo, Mickaël Péron, Margaux Mathieu-Resuge, Philippe Soudant, Fabienne Le Grand, David Mazurais, Marie Vagner

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General Introduction

1. Marine ecosystems and global change

Marine ecosystems play an essential role in the food provision, economy, cultural heritage and global climate in the world. They are characterized by a wide diversity of organisms that form complex interactions and ecological relationships (Costello et al. 2010). They are a keystone of human food security, providing a substantial portion of protein intake for the human population, up to more than 50% for some countries (FAO 2020). Marine ecosystems have also a great socio-economical value as they contribute to the global economy through various sectors including fisheries or tourism (Rogers et al. 2014). Collectively, these sectors form the so-called "ocean economy", estimated to be worth around 1.5 trillion USD per year, with projections indicating this may double by 2030 (OECD 2016). The importance of marine ecosystems extends to global climate regulation. The oceans serve as a major carbon sink, absorbing approximately a quarter of the carbon dioxide generated by human activities annually (Ciais et al. 2013). This process of carbon sequestration is largely driven by photosynthesizing marine organisms (like phytoplankton), at the basis of the marine food web, thus helping mitigate climate change impacts (Sabine et al. 2004). These photosynthesizing marine organisms are also of pivotal importance for the world's oxygen production, contributing to about 50% of the worldwide production (Field et al. 1998).

Climate change has always been a present phenomenon throughout Earth history (Huggett 2012). However, the current rate and magnitude of the recent change, driven primarily by anthropogenic activities, are unprecedented (Cook et al., 2016; IPCC, 2013). According to the Intergovernmental Panel on Climate Change (IPCC), the global average surface temperature has risen by approximately 1.0°C since the pre-industrial era (1850-1900), with most of this warming occurring in the last 35 years (IPCC, 2013). It is also projected that warming will continue throughout the 21st century and beyond, with a further increase in global average surface temperature of 1.5–4.5°C by 2100, compared with the pre-industrial era, under a range of future CO₂ emission scenarios (IPCC, 2013). These changes directly affect, among others, marine life with warming causing species to shift towards higher latitudes or deeper waters to seek for new optimal thermal habitats (Pinsky et al. 2013). Additionally, warmer waters increase the prevalence and severity of disease outbreaks and harmful algal blooms, with potential cascading impacts on the food web (Harvell et al. 2002). Simultaneously, the

amount of carbon dioxide in the atmosphere has increased by over 40% since pre-industrial times, from about 280 parts per million (ppm) to over 400 ppm in recent years (Keeling et al. 2001). As a result, ocean pH decreased from 8.11 to 8.06 from 1980s to 2016 corresponding to a 30% increase in acidity and occurred at a pace a hundred times faster than natural pH variations (IPCC 2023). As an example of consequences, lower pH levels hinder the calcification process of many marine organisms (Wood et al. 2008) or modify fish neurosensor responses (Heuer and Grosell 2014) which could imply thereby threatening the stability of marine ecosystems (Doney et al. 2009). From variations in temperature, pH, salinity, and nutrient availability, to the presence of predators, competitors, and symbiotic partners, each parameter of an organism's habitat can significantly impact its fitness (e.g survival), functions (e.g physiology, behavior, reproduction) and distribution within the ecosystem (Parmesan and Yohe 2003) (Figure 1).

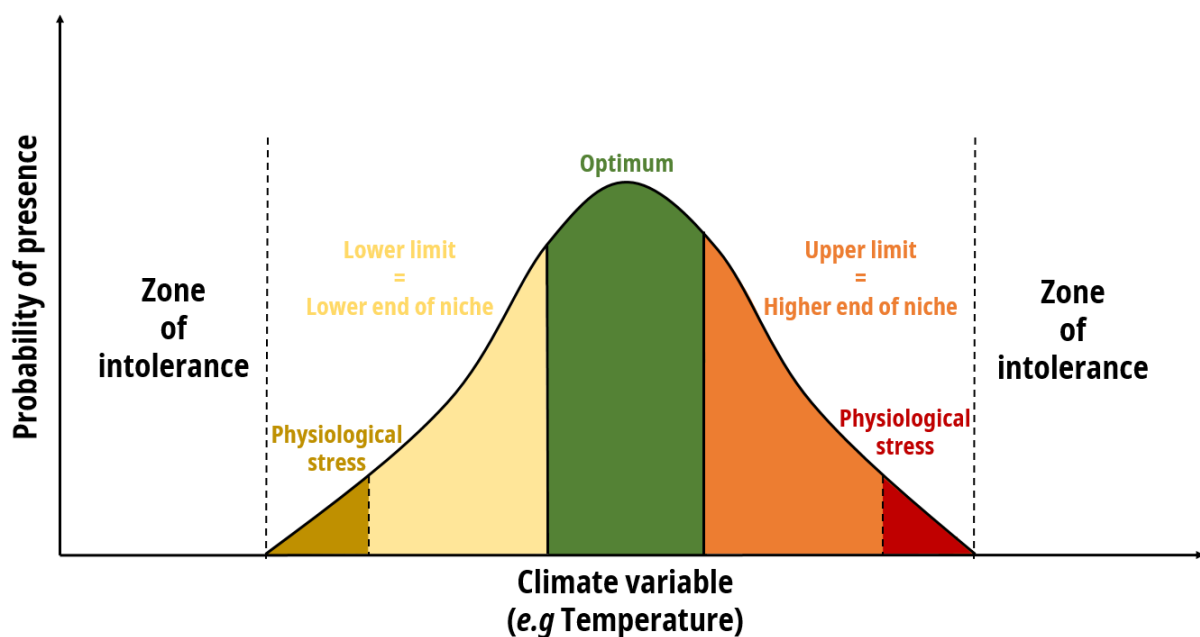


Figure 1: Schematic representation of a species distribution within a niche depending on a climate variable.

Climate change and other anthropogenic stressors can drastically alter the characteristics of habitats. From changing water temperatures to ocean acidification, habitat destruction or trophic food web modifications, these alterations can directly affect the survival and development of marine organisms. The shifting baseline of what constitutes a "favorable" habitat poses new challenges to these organisms, potentially leading to changes in population

dynamics and community structures over time (Cheung et al. 2009). As a result, not only individuals but also populations, communities and ecosystems are expected to shift to address these challenges (Cheung et al. 2008; Bellard et al. 2012).

1.1. Strategies facing environmental modifications

Facing changing environmental conditions, the possibilities for organisms are limited and can be summarized into three main categories: Migrating, adapting through natural selection or acclimating to a new environment. Migration involves the movement of species away from unfavorable conditions towards areas where environmental conditions match their tolerance limits. This type of response is often observed in mobile organisms that have the ability to relocate rapidly over large spatial scales in response to changing environmental conditions. This has been observed for several fish species that changed their distribution area in response to the recent warming in the North Sea (Perry et al. 2005). The Atlantic cod (*Gadus morhua*) populations in the Barents Sea have expanded northwards and eastwards as a result of a warming ocean, leading to a restructuration of the marine communities in these areas (Fossheim et al. 2015). However, not all species are capable of such migrations. Some organisms are limited by their physiological abilities to tolerate changes in temperature, salinity, or other key environmental factors, while others might be hindered by geographical constraints, such as coastal features that limit dispersal.

Adaptation involves genetic changes in populations over generations, driven by natural selection. This process requires time, often across many generations, and hence may be insufficient to keep pace with the current rapid changes in climate. These genetic adaptations to climate change are relatively scarce (Merilä and Hendry 2014). For example, evolutionary changes in the thermal tolerance of marine species have been observed in experimental settings (Barrett and Schluter 2008) and in some wild populations, genetic changes in response to climate change have been documented (Kovach et al. 2012; Kelly and Hofmann 2013). However, as the rate of environmental change accelerates, there may simply not be enough time for natural selection to effect significant changes.

While the threats posed by global change to marine ecosystems and the organisms that make it up are profound, it is crucial to note that organisms are not passive recipients of their environment. Instead, they are dynamic entities capable of adjusting their physiology,

behavior, and life history strategies in response to environmental change. This phenomenon, termed as 'phenotypic plasticity', refers to the ability of a single genotype to produce different phenotypes in response to varying environmental conditions (Pigliucci 2005). Phenotypic plasticity is a key mechanism that allows organisms to cope with and survive in changing environments and has been proven to be the main response of organisms facing climate change (Gienapp et al. 2007; Merilä and Hendry 2014). This plasticity can manifest in a multitude of ways across different levels of biological organization. Organisms can display local adaptations, a phenomenon where populations of the same species develop distinct traits in response to the specific conditions of their local environment like temperature, salinity or anthropogenic stressors (Schulte 2007; Barrett et al. 2008). Under the stress induced by environmental changes, a multitude of parameters can be affected such as swimming performances (Johansen and Jones 2011), morphology (Chapman et al. 2009) or growth (Sandblom et al. 2016). The extent and nature of these changes are governed by the organism's genotype, the range of variability of the environmental factors it encounters, and the interaction between the two (Ghalambor et al. 2007).

One aspect of phenotypic plasticity is developmental plasticity, which involves changes in an organism's developmental trajectory in response to environmental cues. Temperature is an important factor that can shape and affect organisms, especially poikilotherms, during their development and can influence their growth (Jonsson et al. 2012), morphology (Tåning 1952), behavior (Jonsson and Jonsson 2019) and even muscle fiber recruitment (Johnston et al. 2009; Campos et al. 2013; Bizuayehu et al. 2015). Another potential mechanism for rapid responses to climate shifts is the transgenerational plasticity (TGP). This mechanism lies in the ability of parents to influence the phenotype of their offspring in response to environmental cues, independent of genetic changes (Mousseau and Fox 1998; Uller 2008). Such responses can be found across a variety of taxa and has been identified as a potential key player in how populations might respond to climate change (Salinas et al. 2013; Donelson et al. 2018). TGP has been observed in response to various environmental changes, including alterations in temperature (Salinas and Munch 2012), pH (Murray et al. 2014), and salinity (Heckwolf et al. 2018). As a result, the offspring are better adapted to changing environments and show increased tolerance. Yet, the benefits of TGP are likely to be context-dependent, depending on the specific characteristics of the environmental change, the life history traits of the

species, and the costs associated with the plastic responses (Bonduriansky et al. 2012; Salinas et al. 2013). Plastic responses have limits beyond which the organism can no longer adjust to environmental changes (Nussey et al. 2007). Some changes imposed by climate change may be too swift or extreme for phenotypic plasticity to keep pace (Lavergne et al. 2010; Salamin et al. 2010). In addition, phenotypic plasticity implementation can have associated costs such as energy expenditure, reduced performance in other traits, or even maladaptive responses, potentially compromising the overall fitness of the organism (DeWitt et al. 1998).

1.2. Juvenile stage and coastal systems

A key life stage in organisms that can be heavily impacted by environmental changes is the juvenile phase. It acts as a pivotal transitional stage between the larval and adult life stages (Werner and Gilliam 1984; Chambers and Trippel 1997). Mortality rates are usually high during the juvenile stage due to predation and decrease as the fish grows (Houde 1987). Rapid growth of young individuals is thus crucial to exit the most vulnerable size classes rapidly and avoid predation (Sogard 1997). The juvenile stage involves numerous physiological changes, is often marked by a shift in habitat, diet and behavior (Gibson 1994; Sogard 1997), and is a central point where organisms shift from immature to being capable of reproduction. These processes shape the successful transition to adulthood and have profound implications for population dynamics (Shepherd and Cushing 1980; Fuiman and Werner 2009). Changes in environmental conditions can significantly influence the juvenile phase. For example, increased water temperatures can alter the timing of sexual maturation, disrupt growth patterns, and increase susceptibility to diseases (Pankhurst and Munday 2011)

Estuaries and coastal systems are hotspots of productivity and nurseries for many aquatic organisms and especially juveniles (Beck et al. 2001). They are positioned at the interface of land and sea and subject to fluctuating environmental conditions such as salinity, temperature, and nutrient level (McLusky and Elliott 2004). These ecosystems rely on the regular influx of nutrients such as nitrogen, phosphorus, and various trace elements, which underpin the primary production, fuel the food web, and sustain the rich biodiversity of these areas (Childers et al. 2000; Cloern and Jassby 2010). Their dynamic nature is significantly influenced by both marine and terrestrial inputs, creating an environment that demands adaptability from its inhabitants (Elliott and Whitfield 2011). Considering their positioning,

estuaries and coastal systems are in the grip of climate change influencing their physico-chemical properties (Statham 2012; Robins et al. 2016). These areas are the most impacted by anthropogenic activities and are affected by runoff of pollutants (e.g polycyclic aromatic hydrocarbons, heavy metals) and nutrients into coastal waters as another source of stressors for organisms (Vitousek et al. 1997; Syvitski et al. 2005; Halpern et al. 2008).

1.3. Long chain omega-3 fatty acid production and transfer within the trophic web

In marine and estuarine ecosystems, primary producers gather a broad range of organisms, including among them photosynthetic microalgae, protists and bacteria (Simon et al. 2009; Caron et al. 2012; Biller et al. 2015). These organisms support the production of most ecosystems (Lindeman 1942). Primary production is defined by the production of organic matter from mineral matter (Falkowski and Knoll 2011), and photosynthetic organisms are the largest contributors to this production (Falkowski and Raven 2007). Phytoplankton plays a crucial role in producing essential nutrients, especially long chain polyunsaturated fatty acids (LC PUFA). These fatty acids are vital components of cell membranes in most organisms, including fish and humans, which have a limited ability to produce these fatty acids on their own, and then need to obtain them through their diet (Sargent et al. 2003a). LC PUFA are transferred across the food web benefiting to higher trophic levels such up to humans. The fatty acid (FA) composition of membranes is shaping the structure and function of the cell and tissues. They influence whole organism performance and up to higher organizational levels (Arts et al. 2009; Parrish 2013). Unlike terrestrial trophic ecosystems, the marine trophic ecosystem is characterized by high levels of long chain PUFA of the n-3 type (LC n-3 PUFA, also called LC omega-3 PUFA). They are composed of a long carbon chain (≥ 20 atoms of carbon) and at least ≥ 3 double bonds. Among these LC n-3 PUFA, eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) are the most present in the cell membranes of most marine organisms (Tocher 2003). In natural environment, diatoms, dinoflagellates, cryptophytes and haptophytes are the main groups producing these molecules (Li-Beisson et al. 2019), with diatoms being the most effective one to transfer these FA through the food chain (Oliver et al. 2020). In both mammals and fish, EPA and DHA play a crucial role in ontogenesis, growth, and survival, as well as in the development of neuronal and cognitive functions (vision, nervous system) (Simopoulos 1991; Sargent et al. 2003). They are also

significant in prevention and resistance to neurodegenerative or cardiovascular diseases (Hooper et al. 2004; Calon and Cole 2007; Lavie et al. 2009; Avallone et al. 2019)

EPA is a precursor to eicosanoids, which are cellular mediators that act on the nervous system, osmoregulation and inflammatory responses (Bell et al. 1996; Tocher 2003). In some fish species, schooling behavior, brain development, and visual acuity are parameters affected by nutritional DHA (Ishizaki et al. 2001; Benítez-Santana et al. 2007).

The ongoing climate change that alters the physical and chemical properties of the oceans is also affecting the primary producers (Chavez et al. 2011; Kwiatkowski et al. 2020). Some studies have shown a negative relation between an increase in water temperature and accumulation of EPA and DHA in photosynthetic marine microalgae ((Renaud et al. 2002; Guschina and Harwood 2006; Chen 2012). The last decade, several meta-analyses attempted to predict the impact of changing environmental conditions on the production and distribution of LC n-3 PUFA at a global scale (Galloway and Winder, 2015; Hixson and Arts, 2016). In 2015, Galloway and Winder analyzed more than 200 species from six major taxa and concluded that, at the global scale, the nature of FA availability in aquatic ecosystems would mostly result from the taxonomic composition of the phytoplankton community. They also stated that a community shift towards a domination of cyanobacteria, which do not produce LC n-3 PUFA, is likely to result in lower LC n-3 PUFA production and availability for higher consumers. Cyanobacteria have been shown to be adapted to higher temperature and acidification, contrary to other phytoplankton species, leading to their potential dominance with global change (Paerl and Paul 2012), and thus to a decreasing LC n-3 PUFA availability through the trophic web. This potential global scale depletion of LC n-3 PUFA was also underlined by the meta analysis of Hixson and Arts (2016) who gathered more than 950 FA profiles from six major groups of both marine and freshwater phytoplankton. Because of an increase of sea surface temperature of 2.5°C, they predicted a reduction in the production of EPA and DHA by 8.2% and 27.8%, respectively. A global decrease in the production of LC n-3 PUFA at the base of the food web could limit the availability of these molecules for consumers, and thus have cascading effects on their physiology, impacting thus aquatic and even terrestrial ecosystems that depend on the fluxes of aquatic biomass (Gladyshev et al. 2013).

2. LC n-3 PUFA biological functions

2.1. Lipids: Definition and functions

Lipids are a diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents and general insolubility in water (Fahy et al. 2005). They are particularly present in marine organisms where they can represent up to 20% of the organic carbon (Rullkötter 2006) and up to 60% for some primary producers (Fernández et al. 1994).

Lipids can be divided in two classes: (1) reserve lipids or neutral lipids (NL), commonly found as triglycerides, act as energy stores within an organism and (2) membrane lipids (or polar lipids; PL) that are primarily phospholipids. Lipids are the most efficient source of ATP compared to the other macronutrients with 39.5 kJ.g⁻¹ compared to 23.7 and 17.2 kJ.g⁻¹ for proteins and carbohydrates, respectively (NRC 1993). Carnivorous fish are particularly dependent on lipids to fulfill their energy requirements as they have a low ability to digest carbohydrates (Wilson 1994). Energy is generated from lipids in the form of ATP via mitochondrial β -oxidation (Sargent et al. 1989). Depending on the fish species, reserve lipids can be stored in various tissues such as the liver, muscle or adipose tissue (Bruslé and Anadon 1996; Weil et al. 2013). Polar lipids are crucial for maintaining the integrity and fluidity of membranes and play essential roles in various cellular functions (Wang and Tontonoz 2019). PL are generally characterized by a hydrophilic head and two hydrophobic fatty acyl tails. They form a bilayer structure that is the core architectural feature of all cellular membranes along with sterols (van Meer et al. 2008; Wang and Tontonoz 2019). Membrane lipids are not just static structural elements; they play dynamic roles in cellular signaling, transport, and function (van Meer et al. 2008; Wymann and Schneiter 2008).

2.2. Fatty acids: Definition and functions

Fatty acids are lipids and can be found both as free fatty acids and as part of more complex lipids like triglycerides and phospholipids. They are carboxylic acids with an aliphatic chain composed from 4 to 28 carbon atoms. The nomenclature of fatty acids is typically based on the number of carbon atoms in the chain, the number of double bonds, and the position of the double bonds. They can be represented as "C:Xn-Y," where C represents the number of

carbon atoms, X the number of double bonds, and Y indicates the position of the first double bond counting from the methyl end. Long chain (LC) FAs are composed of 20 or more carbons. Fatty acids can be categorized into different families based on the number and position of double bonds. The primary families include saturated fatty acids (SFA) with no double bonds, monounsaturated fatty acids (MUFA) with one double bond, and polyunsaturated fatty acids (PUFA) with two or more double bonds. The types of fatty acids are further classified based on the location of the first double bond from the methyl end (CH₃). The main classes include omega-9 (n-9), omega-6 (n-6), and omega-3 (n-3) fatty acids, depending on which carbon (3rd, 6th or 9th) the first double bond is positioned from the terminal CH₃. For example, DHA (22:6n-3) is a LC n-3 PUFA of 22 carbon atoms, with 6 double bonds, and the first one is located on the 3rd carbon atom from the terminal CH₃. Arachidonic acid (ARA, 20:4n-6) is a LC n-6 PUFA, with 20 carbon atoms, 4 double bonds, and the first one is located on the 6th carbon atom from the terminal CH₃ (Figure 2).

2.3. Importance and benefits of LC PUFA

In vertebrates, LC PUFA are fundamental to the structure and function of cellular membranes as they are their main components (Vaskovsky 1989). As mentioned in paragraph 1.5.1, these fatty acids, incorporated into phospholipids, provide, among other, fluidity and permeability to membranes, influencing their behavior and properties (Sargent et al. 2003; Eldho et al. 2003). The presence of LC PUFA modifies the permeability of the membrane to various ions and molecules (Bell et al. 1986). It influences the function of membrane proteins such as ion channels, transporters, and receptors, thereby affecting cellular signaling and communication (Murphy 1990). They are also known to influence the function of membrane-bound enzymes and receptors, playing a critical role in various physiological pathways (Hulbert 2007). In humans, DHA is involved in maintenance of neural, cognitive and reproductive functions, as well as in the reduction of cardiovascular and neurodegenerative diseases (Swanson et al. 2012; Ghasemi Fard et al. 2019)

EPA and ARA are involved in the inflammatory responses through the production of eicosanoids but also in growth in both fish and humans (Wall et al. 2010; Xu et al. 2022).

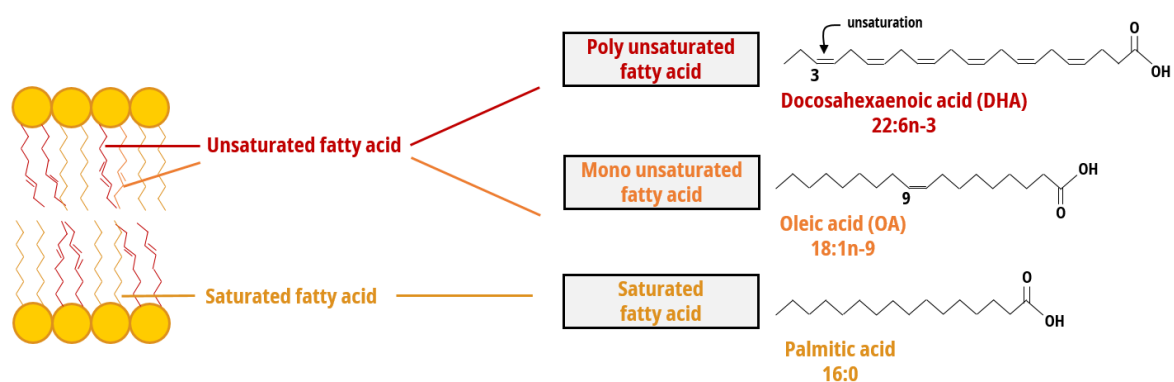


Figure 2: Schematic representation of membrane lipids and fatty acids.

2.4. Biosynthesis of LC PUFA

Some FA can be synthesized de novo by vertebrates, including fish. This phenomenon, defined as lipogenesis, mostly occurs in the liver, and can produce FA from the n-9 series such as oleic acid (18:1n-9), which is preferentially used for energy storage or production (Bell et al., 2004; Henderson and Sargent, 1985; Sargent et al., 2003; Tocher, 2003). The biosynthetic pathways of FA is based on their specific series, (i.e n-9, n-6, or n-3) (Figure 3), and involve two major families of enzymes, i.e. desaturases and elongases (Castro et al. 2016). In vertebrates, LC PUFAs from both n-3 and n-6 families are synthesized from their respective precursor fatty acids, which must be obtained through the diet (Arts and Kohler 2009a). These precursors are linoleic acid (18:2n-6, LA) for the n-6 series, and α -linolenic acid (18:3n-3, ALA) for the n-3 series (Figure 3). Longer chain PUFA like EPA or DHA can then be obtained by desaturation (adding of double bond) and elongation (adding of carbon atom) from these precursors. However, in organisms of high trophic levels, like fish, these conversion rates are usually low and depend on a multitude of factors, such as the species considered and its environment (Tocher 2015).

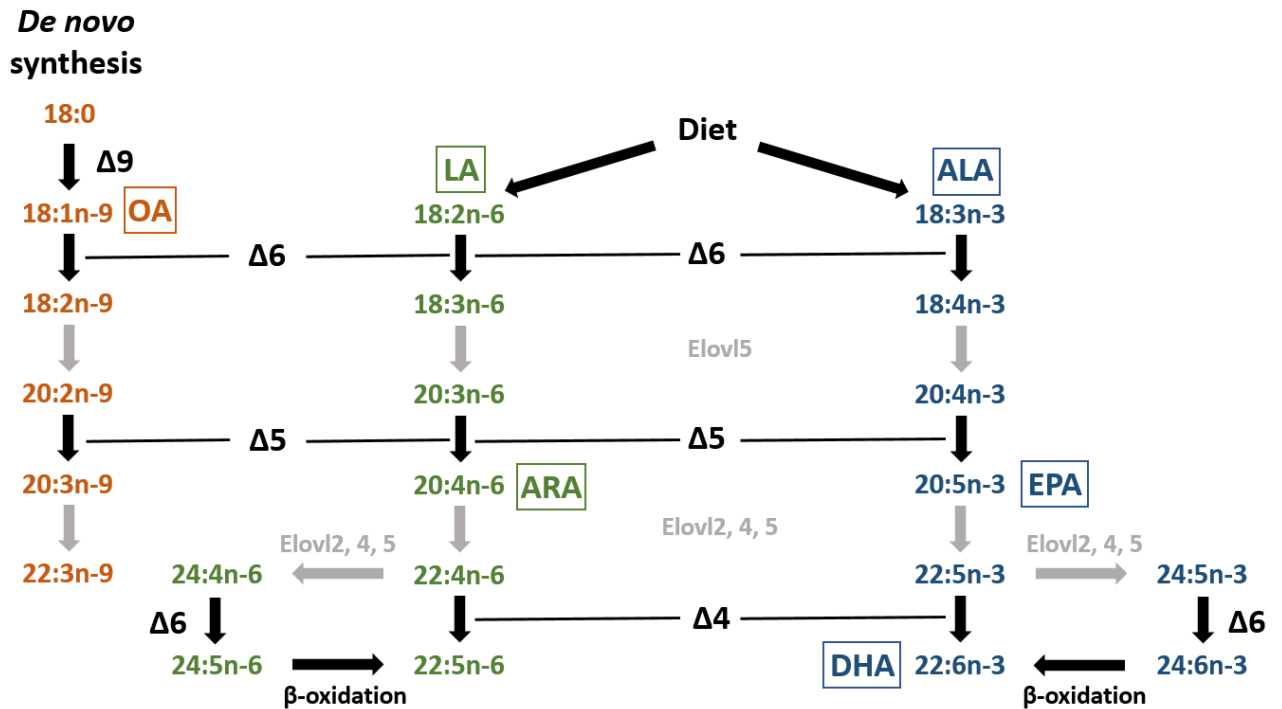


Figure 3: Schematic representation of the biosynthetic pathways of LC PUFA of the n-9 (orange), n-6 (green) and n-3 series (blue) in teleosts. Black and gray arrows between two FA represent reactions catalyzed by fatty acyl desaturase (Δx) and fatty acyl elongase (Elovlx) activities, respectively. ALA: α -linolenic acid, ARA: Arachidonic acid, DHA: Docosahexaenoic acid, EPA: Eicosapentaenoic acid, LA: Linoleic acid, OA: Oleic acid. Modified from Garrido et al. (2019).

The efficiency of FA biosynthetic pathways, depending on the activity of the desaturases and elongases, varies among species, and is influenced by various biotic and abiotic factors, such as temperature or diet.

Contrary to marine fish, freshwater fish species have generally been found to have a higher capacity for LC PUFA biosynthesis (for review, see Tocher, 2010). This ability could be an adaptation to their environment, where LC PUFA are less available in the trophic environment due to a lesser primary production of these PUFA, leading to the necessity for more efficient synthesis pathways (Turchini et al. 2009; Hixson et al. 2015). The larger disponibility of LC PUFA and particularly of the n-3 series in the marine environment would then induce a lack of selective pressure on the biosynthesis pathways explaining the reduced ability of marine consumers to convert precursors into longer chain FA (Tocher et al. 2006).

In marine fish species, the activity of $\Delta 6$ desaturase (or *fads2*, fatty acid desaturase2; **Figure 3**) is often considered as a limiting step in LC PUFA biosynthesis, as it is the first one (Vagner and Santigosa 2011). Investigations using the hepatocytes from both freshwater and marine fish have revealed differences in the efficiency of the biosynthesis pathways. Freshwater fish such as zebrafish (*Danio rerio*) or Nile tilapia (*Oreochromis niloticus*) have shown an ability to produce 18:4n-3 from the 18:3n-3, as well as EPA and DHA through an activity of $\Delta 6$ desaturase (Tocher et al. 2001), **Figure 3**). Opposite findings were highlighted in marine species such as turbot *Scophthalmus maximus* (Linares and Henderson 1991) or European sea bass *Dicentrarchus labrax* (Geay et al. 2010a).

2.5. Regulation of LC PUFA biosynthesis

In both marine and freshwater fish species, the regulation of LC PUFA biosynthesis is a complex and dynamic process that influences the FA composition of organisms. Global change and/or lower scale variations of environmental parameters may have an impact on this FA composition, as LC PUFA biosynthesis can be regulated by different environmental parameters.

2.5.1. Temperature

The membrane lipids FA composition is regulated by various environmental factors such as temperature, especially in poikilothermic species, like fish, whose internal temperature depends on that of their environment. At higher temperatures, lipid molecules gain kinetic energy, thus membranes become more fluid enhancing permeability and transport across the membrane (Los and Murata 2004). Conversely, at lower temperatures, membranes become rigid, reducing permeability and the activity of membrane proteins. Extremely low temperatures can lead to a gel-like phase, impairing cell function (Los and Murata 2004). In response to temperature variations, the fluidity of cell membrane is continuously adjusted with temperature in order to maintain its functional properties. This adjustment of the membrane is called homeoviscous adaptation (Sinensky 1974). When temperature gets colder and membrane more rigid, the membrane has to become more fluid, and this is possible with the incorporation of longer and more unsaturated fatty acids FA (Hazel et al. 1992; Lande et

al. 1995; Calabretti et al. 2003; Stillwell and Wassall 2003; Guschina and Harwood 2006) (Figure 2). For example, its 5 double bonds confer to EPA a particular 3D structure and the insertion of this molecule in cell membranes creates folds in their structure, preventing close packing of fatty acid chains. This results in an increased membrane fluidity, allowing for greater flexibility and adaptability in response to environmental changes (Stillwell and Wassall 2003). On the opposite, when temperature gets warmer and membranes more fluid, PUFA or MUFA are replaced by SFA, reducing the number of double bonds, and readjusting the membrane fluidity (Skalli et al. 2006).

Temperature fluctuations can induce changes in the activity of the key enzymes of LC PUFA biosynthesis: desaturases and elongases (De Torrenco and Brenner 1976; Ruyter et al. 2003). Under low temperatures, desaturation and elongation capacities were found to be enhanced in common carp (*Cyprinus carpio*), leading to an increase in unsaturation of the membrane FA to optimize fluidity (Wodtke and Cossins 1991). On the contrary, in warmer conditions, a decrease in $\Delta 6$ desaturase activity has been observed in rainbow trout (*Oncorhynchus mykiss*, Tocher et al., 2004). This temperature-dependent regulation of FA biosynthesis can vary significantly among species and environments, with freshwater fish often displaying higher biosynthetic capabilities compared to marine species.

2.5.2. Salinity

Salinity is also a parameter that can influence the biosynthesis of LC PUFA. It has been shown that, in a high salinity environment, anadromous species like salmonids had a reduced capacity to biosynthesize LC PUFA (Zheng et al. 2005b). This salinity regulation has also been shown in marine fish (*Signatus canaliculatus*, (Xie et al. 2015) and freshwater fish (*O. niloticus*, You et al., 2019). This regulation may be required for membrane lipids remodeling associated with osmoregulatory responses (Fonseca-Madrigal et al. 2012). It has recently been shown in *S. canaliculatus* that *fads2* ($\Delta 6$ desaturase) expression was regulated by salinity, both at a transcriptional and post-transcriptional levels (Xie et al. 2021). However, and like for temperature, the effect of salinity on LC PUFA biosynthesis depends on the considered species and salinity levels (Monroig et al. 2018a).

2.5.3. Diet

The regulation of LC PUFA synthesis in fish by nutritional factors has been widely investigated, particularly in farmed fish species (Mourete et al. 2005; Turchini et al. 2009; Tocher 2015; Alhazzaa et al. 2019).

Numerous studies have examined the biosynthesis abilities of fish within the context of aquaculture, as the use of fish oil (FO), presents a sustainability issue. FO are derived from marine raw materials and naturally rich in LC n-3 PUFA, causing overfishing of some species to fuel the aquaculture industry (FAO 2020). This sustainability issue led to the replacement, at targeted periods of the fish growth, of FO by vegetable oils (VO) such as soybean, linseed or canola oils, that display a lower LC n-3 PUFA content than FO, but a higher amount of their precursors (Tocher 2010a). In fish fed oil from transgenic *Camelina sativa* as an alternative source of EPA, DHA has been observed to participate in feedback control mechanisms. This involvement of DHA results in the suppression of the expression of genes related to the biosynthesis of LC PUFA, including the gene for $\Delta 6$ desaturase (Betancor et al. 2015, 2016). This suppressive mechanism is crucial as it helps to regulate and maintain the appropriate levels of LC PUFA in the fish, ensuring a balance in the biosynthesis of these essential fatty acids. Although the LC PUFA biosynthesis is enhanced through higher activity of elongases and desaturases in salmonids fed VO, the tissue levels of LC PUFA remained lower than fish fed FO (Tocher 2009, 2015). In marine fish, European sea bass subject to a dietary deficiency in LC n-3 PUFA at the larval stage was able to up-regulate, at molecular level, the PUFA synthesis pathways at the juvenile stage (Vagner et al. 2007b).

2.6. Dietary fatty acids modulate fish physiological and behavioral performances

This modification in the FA quality of dietary sources, consequently modifying FA composition, may have significant implications on the growth and other biological performance of the fish (Figure 4, Figure 5).

As said earlier, dietary LC n-3, PUFA content significantly influences cell membrane composition, affecting cell functionality. In cascade, these dietary content impacts tissue composition and functionality, as efficient cell function is crucial for tissue responsiveness and energy utilization. In consequence, dietary LC n-3 PUFA content influences biological

performance at the individual level, such as growth, locomotion, energy metabolism, and behavior (Pilecky et al. 2021). Thus, dietary LC n-3 PUFA content plays a pivotal role in determining the overall health and performance of an organism (**Figure 4, Figure 5**) (Pilecky et al. 2021; Závorka et al. 2023).

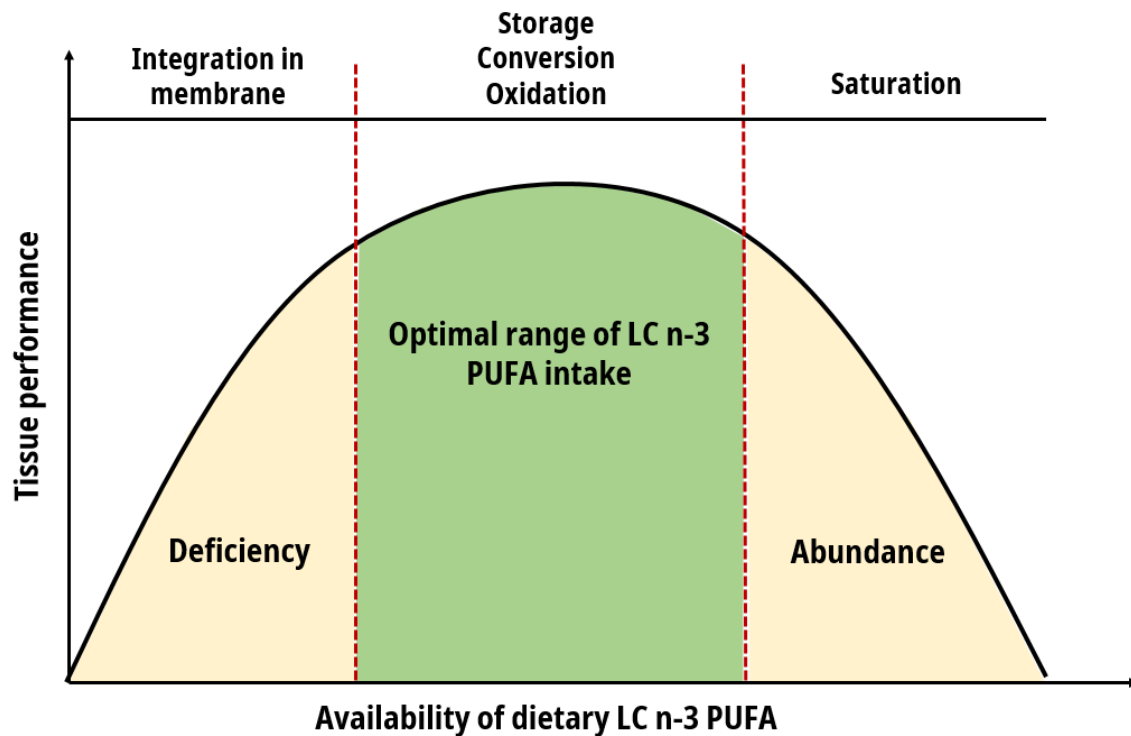


Figure 4: Schematic representation of the hypothesis linking LC n-3 PUFA and tissue performance. (Adapted from Závorka et al., 2023).

Understanding the physiological and behavioral responses of aquatic organisms to environmental factors, such as LC PUFA availability is fundamental for both aquaculture and conservation practices, especially in the context of global change. These responses determine the maintenance of population, and in cascade, the energy and matter fluxes within the whole ecosystem (**Figure 5**). Growth performance is a key indicator of the nutritional and health status of organisms, providing insights into their energy acquisition and utilization (Jobling 1995). Analyzing swimming performance helps in understanding the organism's locomotory efficiency and endurance, which are crucial for their survival, foraging, and escape from predators (Plaut 2001). Behavioral performance studies offer valuable insights into the organisms' responses to environmental stimuli, social interactions, and stressors, predator-prey relationships, which are fundamental for their survival and reproduction (Reebs 2000). Together, these performance metrics provide a comprehensive understanding of the

organism's physiological state and fitness, aiding in the development of conservation strategies and sustainable aquaculture practices.

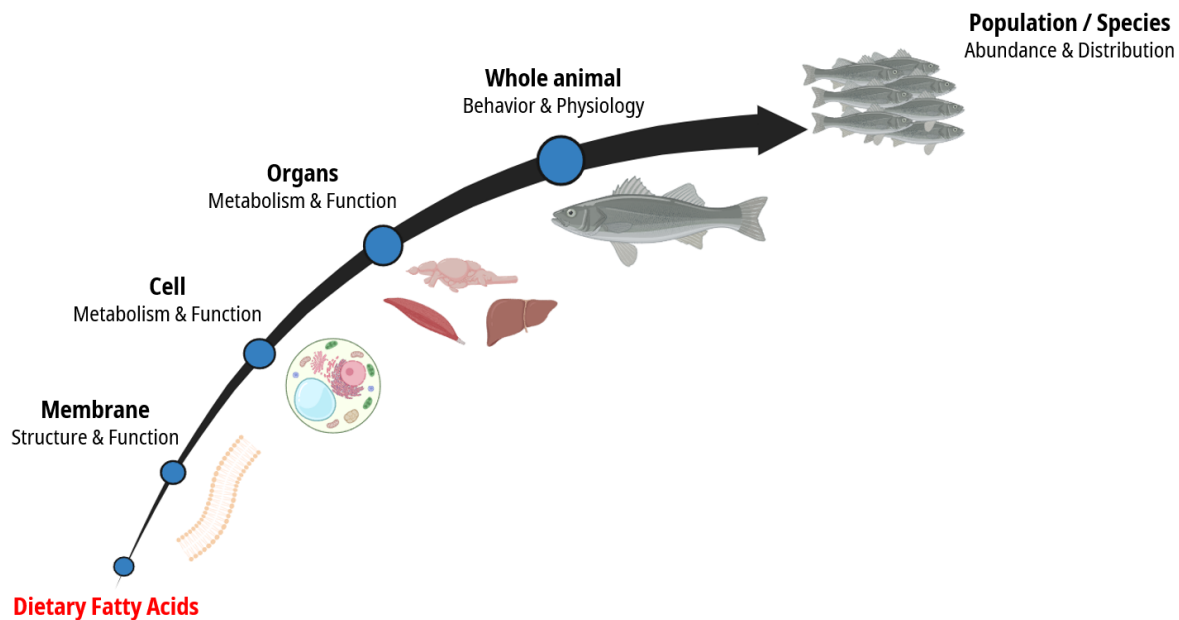


Figure 5: Schematic representation of the chain of consequences from dietary FA to the population dynamics.

2.6.1. Growth performance

The effects of dietary FA on growth performance has been extensively studied in many fish species including freshwater species like the Murray cod (*Maccullochella peelii*; Turchini et al., 2011), Nile tilapia (*O. niloticus*; Trushenski et al., 2009), marine species such as European sea bass (*D. labrax*; Torrecillas et al., 2017), turbot (*S. maximus*; Peng et al., 2014; Regost et al., 2003), gilthead sea bream (*Sparus aurata*; Montero et al., 2003; Ofori-Mensah et al., 2020), and anadromous species such as Atlantic salmon (*Salmo salar*; Metochis et al., 2017). These studies were mostly involved in the context of aquaculture. Usually, freshwater and anadromous species can withstand a higher replacement of FO by VO due to their higher ability to convert the precursors included in it, into LC n-3 PUFA. In Atlantic salmon, a total replacement of FO by canola oil did not affect the growth performance over 17 weeks (Bell et al. 2001). The same results were observed in rainbow trout (*O. mykiss*) fed VO, but the substitution generated a modification of flesh FA composition, altering its nutritional quality

for human consumption (Fonseca-Madrigal et al. 2005; Yildiz et al. 2015). In European sea bass, studies have led to an estimation of the requirements for LC n-3 PUFA in the diet of about 0.7% of dry matter (DM) (Skalli and Robin 2004). Below this threshold, the growth of the juvenile sea bass decreased, and traits like immune response or disease resistance can be affected (Torrecillas et al. 2017c). DHA-induced promotion of growth performance through white muscle hyperplasia and muscle fiber development has been shown in blunt snout bream and grass carp *Ctenopharyngodon idella* (Wang et al. 2020; Ji et al. 2024).

2.6.2. Locomotion

Other integrative traits (e.g depending on a range of physiological functions) can be affected by dietary LC n-3 PUFA in fish, such as swimming performance.

Dietary LC n-3 PUFA content influences the structure and function of cells, including that of muscles (McKenzie 2001; Vagner et al. 2014), which in turn could impact muscle tissue performance and further integrative traits like swimming (Chatelier et al. 2006). In addition, muscular performance relies on metabolic fuel, which is mostly provided by lipids as TAG (Weber 2011).

Swimming is the primary mode of locomotion for a majority of fish species. It dictates how fish interact with their environment (Haro et al. 2004) and plays a vital role in many aspects of their life, such as foraging (Pimentel et al. 2016), escaping from predators (Blake 2004), reproduction (Palstra and van den Thillart 2010) or migration (Dittman and Quinn 1996). It is overall a main determinant of survival for many fish species and a major integrative trait affecting the fitness of individuals (Reidy et al. 2000; Plaut 2001). Swimming is dependent on a range of physiological factors such as ventilation, oxygen transport and muscle contraction making it an indicator of an animal's physiological suitability (Nelson 1989).

The type of swimming of a fish can be classified based on the duration and the intensity of the effort, as well as on the muscle type (red or white) and energy substrates involved (Beamish 1978). White muscle, making up a large portion (up to 50%) of the total muscle mass, is primarily involved in short, rapid, and powerful bursts of activity due to their fast-twitch fibers (Venugopal and Shahidi 1996; McKenzie 2011). These fibers have a low myoglobin content and primarily rely on anaerobic metabolism for energy production, making them efficient for

quick movements but susceptible to fatigue. Red muscle, on the other hand, is located near the lateral line and is essential for sustained, aerobic swimming (Bone 1979; McKenzie 2011). These muscles contain slow-twitch fibers with high myoglobin content, allowing for efficient oxygen storage and utilization. The high vascularization in red muscles supports aerobic metabolism, providing the endurance needed for continuous swimming. If a fish swims at its top speed for less than 20 seconds, this is known as "burst swimming speed". If the fish keeps up its speed for a duration ranging from 20 seconds to 200 minutes, it is termed "prolonged swimming speed". Lastly, if the fish maintains its speed for more than 200 minutes, it is called "sustained swimming speed" (Brett 1964; Webb 1975; Beamish 1978). During prolonged swimming, when the aerobic capacity of red muscle is reached and the metabolic demand for oxygen and nutrients is exceeded, white muscle and anaerobic metabolism have to be involved. Consequently, swimming speed and anaerobic metabolism increase in parallel (Peake and Farrell 2004).

The maximum aerobic capacity of a fish can be measured experimentally by measuring the critical swimming speed (U_{crit}). U_{crit} , which was first developed almost 50 years ago by Brett, (1964), is determined by increasing the velocity of a water current, in which a fish swims against, by a fixed increment at a fixed time interval. U_{crit} is then, by definition, a measure of prolonged swimming speeds. It is the most convenient and reproducible method to evaluate fish swimming performance and to predict ecological changes on swimming performance, but it is however just an estimation of aerobic capacities, and U_{crit} measurements have to be used while being aware of these limitations (Plaut 2001).

- Swimming performance and dietary FA

Only a few studies have investigated the effect of dietary FA on swimming performance. It has been shown that the quality and quantity of the diet has a direct influence on metabolism and swimming performance (McKenzie 2001). The few studies that investigated the effects of FA on swimming performance show contradictory data because of the variety of species used and the differences in the formulation of feeds. In European sea bass it was found that high U_{crit} was correlated with high levels of MUFA, and especially oleic acid (OA, 18:1n-9), suggesting its utilization as energy substrate (Chatelier et al. 2006). Moreover, Litz et al., (2017) reported a positive correlation between TAG levels and swimming speed in Chinook

salmon (*Oncorhynchus tshawytscha*). McKenzie et al. (1998) reported a lower U_{crit} in Atlantic salmon fed a diet rich in LC n-3 PUFA compared to individuals fed a low LC n-3 PUFA diet enriched with canola oil. However, some studies reported a lower U_{crit} in fish fed low LC n-3 PUFA in the diet. For example, Arctic charr (*Salvelinus alpinus*) fed with a high vegetable oil/fish ratio (3:1) had a decreased U_{crit} compared to individuals fed with a diet containing only fish oil (Pettersson et al. 2010). Wagner et al. (2004) underlined the importance of LC n-3 PUFA/SFA or PUFA/ARA ratios as they were positively correlated with U_{crit} in Atlantic salmon. Golden gray mullet (*Chelon auratus*) fed a diet low (0.2 % EPA + DHA of DM) in LC n-3 PUFA showed a similar U_{crit} as those fed a standard (1.2 % EPA + DHA of DM) amount of these fatty acids (Vagner et al. 2014). However, the ones on the low diet had a higher oxygen consumption per unit distance than those fed the control diet (Vagner et al. 2014).

2.6.3. Metabolic performance

Metabolic rates are (MR) physiological proxies for assessing the bioenergetic demands of swimming fish (Brett 1964; Plaut 2001). MR is a critical physiological parameter that reflects an organism's energy expenditure and, by extension, its energy requirements for various biological processes. It is influenced by many factors such as activity level, temperature, and food intake. MR is often assessed by measuring oxygen consumption (MO_2) of individuals.

Standard metabolic rate (SMR), or basal metabolic rate represents the energy necessary to sustain mandatory activities such as cerebral function, osmoregulation, ventilation (Chabot et al. 2016). It is measured when an animal is in fasting, unstressed, non-reproductive and resting conditions. Adriatic sturgeon (*Acipenser naccarii*) fed a diet rich in SFA for a year had a SMR elevated by more than 30% compared to individuals fed a diet rich in LC n-3 PUFA (McKenzie et al. 1997). The same result was observed in European eel (*Anguilla anguilla*) fed the same diet for 3 months.

Maximum metabolic rate (MMR) is considered as the highest aerobic metabolism possible for an organism to sustain maximal activity. In European sea bass, MMR was decreased when fed a high LC n-3 PUFA compared to fish fed a diet rich in saturated and monounsaturated FA (Chatelier et al. 2006). The difference between MMR and SMR is termed aerobic scope (AS, Fry, 1947), and represents the maximum aerobic capacity (i.e amount of oxygen available) to

sustain non mandatory activities such as swimming, growth, reproduction, digestion (Claireaux and Lefrançois 2007; Norin and Metcalfe 2019). AS was shown to be increased in *C. auratus* fed low LC n-3 PUFA diet (0.2 EPA+DHA DM) coupled with a temperature increased compared to fish fed high LC n-3 PUFA (4.8% EPA+DHA DM) diet (Vagner et al. 2015). Post-exercise recovery can be assessed with the measure of the excess post-exercise oxygen consumption (EPOC) that quantifies the energy and time needed to restore: the tissue and cell storage of oxygen, the balance levels of metabolites such as lactate and glycogen and other metabolic functions such as ionic and osmotic balance (Lee et al. 2003).

2.6.4. Behavioral performances

LC n-3 PUFA and particularly DHA play a pivotal role in maintaining optimal brain function and development as well as cognition (Lauritzen et al. 2001; Bourre 2004). Most studies about brain DHA have focused on mammalian models due to its benefits on human health (Marszalek and Lodish 2005; Lauritzen et al. 2016). Some studies have also shown the importance of DHA for behavior and cognition in fish, as cognitive functions are good indicators of health and development (Masuda et al. 1999; Ishizaki et al. 2001; Martins et al. 2012; Benítez-Santana et al. 2014; Roy et al. 2020). A few studies have also investigated specifically the effects of dietary LC PUFA on the escape response of fish, mostly on larvae. Benítez-Santana et al. (2014) found that DHA rather than EPA positively enhanced sound-induced escape behavior through Mauthner cell activity in *S. aurata* larvae. In a similar study, it was also shown that a reduction in dietary LC n-3 PUFA negatively affects swimming speed and response to a visual stimulus (Benítez-Santana et al. 2007). Larvae of pike perch *Sander lucioperca* fed low levels of DHA had delayed escape response (i.e. a longer latency) and a more anxious profile compared to individuals fed standard levels of DHA (Lund et al. 2014). More recently, the effects of novel oils, with reduced LC n-3 PUFA contents, on escape response of juvenile *S. aurata* were tested (Carvalho et al. 2022). Despite exhibiting elevated synthesis of LC PUFA and increased expression of neurogenesis-related genes, fish that were fed reduced levels of LC n-3 PUFA displayed escape behaviors (including latency, responsiveness, and covered distance) that were comparable to those of control fish.

The escape response, often termed the "startle response", is a behavior exhibited by fish when confronted with sudden and potential threats. This rapid, reflexive movement is typically

characterized by a sudden, fast-paced change in direction, allowing the fish to quickly distance itself from the perceived threat (Domenici and Hale 2019). The underlying neural circuitry for this response is relatively well conserved among fish, with the Mauthner cells, a pair of large neurons located in the hindbrain, playing a pivotal role in initiating the swift escape motion (Korn and Faber 2005). Escape response in teleost fish usually consists of a C-type fast-start (C-start) but there is a multitude of types of escape responses that has been reviewed by Domenici and Hale (2019). The ecological relevance of the escape response is profound. In the predator-rich aquatic environments where split-second decisions can mean the difference between life and death, the efficiency and directionality of the escape response can significantly influence individual survival rates (McCormick et al. 2018). For instance, fish larvae with faster and more directed escape responses have been shown to have higher survival rates in the presence of predators (Fuiman and Cowan Jr 2003). Furthermore, the escape response can be modulated by various factors such as temperature or social behavior. Previous encounters with predators, environmental conditions, and even the physiological state of the fish can influence the latency, speed or direction of the escape (for review, see Domenici, 2010).

2.7. Bottlenecks concerning the effects of FA on physiology and behaviour

Studies on the effects of dietary FA on physiological and behavioral performance in fish are scarce, and sometimes contradictory, indicating the need to increase our knowledge of these effects in a future context of modifying LC n-3 PUFA intakes. For a deeper understanding of the fatty acid metabolism in fishes and to aim to understand how dietary FA modulates physiological performances, this thesis will use a well-known model, which has been studied for many years for aquaculture purposes. However, very few studies have been conducted on sea bass from wild origin (Gourtay et al. 2020) and none on wild-caught sea bass. In addition, domestication of fish has been shown to influence swimming performance and behavior and influences the phenotype (increases growth, reduces stress, regulates immunity) (Beamish 1978; Walker et al. 2005; Milla et al. 2021). Recent studies have stressed the importance of using more ecologically relevant models for experimental studies such as wild-caught fish (Bshary and Grutter 2006; Salena et al. 2021).

2.8. Using non-lethal sample for FA analysis

Understanding the roles and dynamics of FA is of importance for broader physiological, ecological, and nutritional perspectives. Tissue sampling for biological analysis, including FA, is usually done by killing the animal, sometimes for a small piece of tissue (Henderson et al. 2016; Couturier et al. 2020). The sacrifice of an individual also prevents further analysis on the same individual to monitor the changes of the measured parameters over time. With growing awareness of animal welfare and the ethical treatment of research subjects, there is an increasing emphasis on minimizing harm. Thus, there is an increase in the number of studies implementing non-lethal methods to collect tissue from various species, including fish (Busst et al. 2015; Cano-Rocabayera et al. 2015; Jeffries et al. 2021). Nonetheless, this type of method has not yet been used for analyzing FA composition in juvenile fish. The muscle biopsy method has proven its effectiveness as a non-invasive, low-stress method in fish (Henderson et al. 2016). This method has already been used to analyse heavy metal concentrations (Peterson et al. 2004) and stable isotopes (Nelson et al. 2011). This method has been used in juvenile European sea bass to study red muscle mitochondria (Quéméneur et al. 2022). In this species, the biopsy had no impact on the immune response and did not result in a growth deficit (Quéméneur et al. 2022). This type of method could therefore prove useful for monitoring FA composition at individual level over time.

3. Biological model: European sea bass

3.1. Distribution

The European sea bass (*Dicentrarchus labrax*, Linnaeus 1758) belongs to the family of *Moronidae* and has a wide ecological and geographical range of distribution. This is because this species is highly tolerant to great variations of both temperature and salinity. It can inhabit waters ranging from 2° to 32°C (Barnabé 1990; Pauly and Froese 2012) and salinities ranging from 0.5 to 60 (Chervinski 1974). European sea bass is distributed from the south of Morocco to the north of Norway and all over the coast of the Mediterranean Sea (Figure 6). It has also been reported in the Baltic Sea (Bagdonas et al. 2011) and its distribution area is moving more and more towards the North (Pawson et al. 2007).

There is an intraspecific genetic diversity between the populations of the Atlantic and those of the Mediterranean Sea (Fritsch et al. 2007; Pawson et al. 2007; Coscia et al. 2012) . This diversity is most likely due to the closure of the Mediterranean Sea and the physio-chemical differences between the two systems. Despite having a large repartition, the Atlantic populations seem genetically homogeneous unlike the Mediterranean populations that are rather structured (Vandeputte et al. 2019; Faggion et al. 2019).

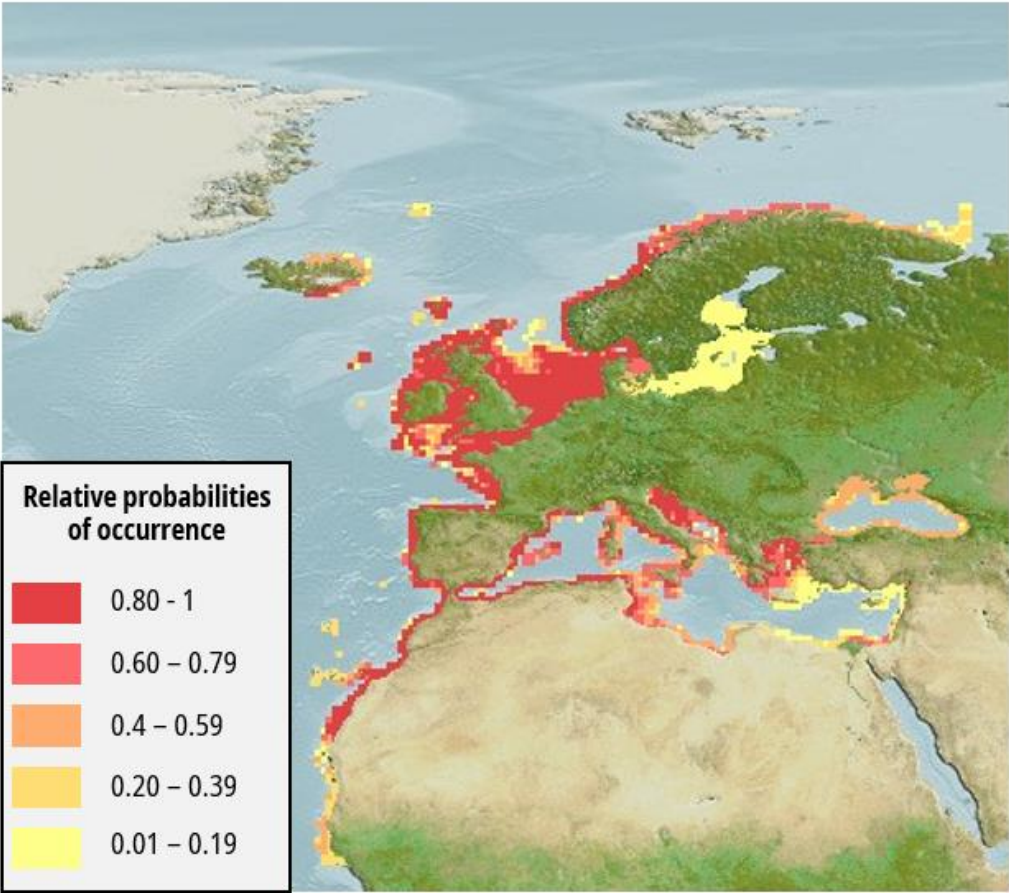


Figure 6: Distribution map of European sea bass (*Dicentrarchus labrax*). The colors indicate the probabilities of occurrence of individuals (modified from AquaMaps, 2019).

3.2. Life cycle and habitat

Adult sea bass usually spawn between December and March but can also spawn up to June (Haffray et al. 2007), although the exact timing can vary depending on the geographical location and water temperature (Pickett and Pawson 1994). In the open sea, females release up to 200,000 eggs/kg, which are then fertilized by males (Haffray et al. 2007). After a few days, the eggs hatch into larvae that are widely dispersed during the first three months of life. The post-larvae then drift towards coastal zones with spring tides and colonize estuaries where they will live as juvenile (Jennings and Pawson 1992). After spending several years in these nursery habitats, the juveniles mature into adults and migrate to open waters (Pickett and Pawson 1994). The age at which sea bass reach sexual maturity can vary, but it's typically around 3-5 years for males and 4-6 years for females, depending on environmental conditions, especially temperature (Figure 7) (Pérez-Ruzafa and Marcos 2014).

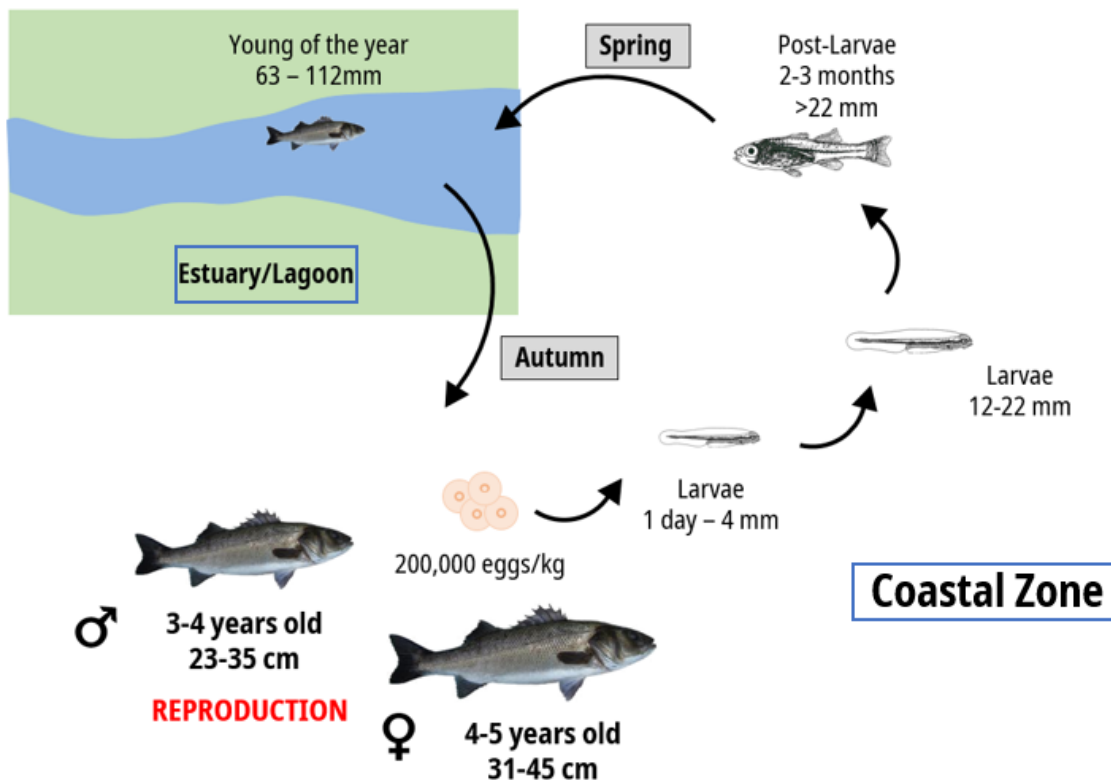


Figure 7: Schematic representation of the lifecycle and migration patterns of European sea bass. (Adapted from Pérez-Ruzafa and Marcos, 2014).

3.3. Feeding ecology

The European sea bass is a keystone in trophic webs, as it is a very opportunistic feeder, foraging on any abundant prey, and a top predator at the adult stage (Boulineau-Coatanea 1969; Spitz et al. 2013). The size of individuals is the main driver of the prey preferences of *D. labrax*. Small individuals typically prefer small prey like *Mysidacea* (Moreira et al., 1992) but also feed on a wide range of benthic organisms such as amphipods, isopods or decapods (Kennedy and Fitzmaurice 1972; Pickett and Pawson 1994; Pérez-Ruzafa and Marcos 2014). When the size of the fish increases, a shift in the diet occurs with an increase in the proportions of fish in the diet and a decrease in the crustaceans/fish ratio (Abdel-Hakim et al. 2010). At adult size, this is even more marked as sea bass will mainly target small pelagic fish (Spitz et al. 2013).

3.4. Aquaculture and fisheries

The European sea bass is a renowned species along all the European coasts. It is among the most abundant and exploited fish species in Europe. It is a species prized by professional and recreational anglers and has increased in popularity since the 1970s (Barnabe et al. 1976). This species is considered as a pioneer in aquaculture of marine fish and was the first marine species raised in intensive conditions (Girin 1979). Over the years, the proportions of aquaculture production over captures have considerably increased. Indeed, in 2000, sea bass total production represented 70,260 T/year with 14% as captures (FAO 2020). This production has more than tripled in 20 years with 249,470 T/year and 2% as captures (FAO 2020). This popularity has led to make the European sea bass one of the most studied fish species with over 500 published papers per year (Pérez-Ruzafa and Marcos 2014).

3.5. The estuary ecosystems

The estuary ecosystems are essential 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). 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 (Ménésguen et al. 2018). They can exhibit broad differences in temperature, salinity or

nutrient availability that modulate the communities within the estuary (Selleslagh et al, 2009). They 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 (Lefebvre et al. 2009; Teichert et al. 2016; Ménesguen et al. 2018).

3.6. Fatty acid research on European sea bass

Numerous studies have investigated the effects of dietary FA in European sea bass, mostly for a production purpose (Castro et al. 2016; Torrecillas et al. 2017a, b; Campos et al. 2019; Betancor et al. 2021a). In addition, European sea bass subject to a dietary deficiency in LC n-3 PUFA during the larval stage have been shown to be able to up-regulate, at molecular level, the PUFA synthesis pathways at the juvenile stage (Vagner et al. 2007a, 2009). However, all these studies were conducted on domesticated fish, which, as previously discussed (section 2.7) can have different genotypes and phenotypes compared to their wild counterparts.

4. Thesis objectives

In the context of a modification of the nutritional availability of LC n-3 PUFA with global change in marine ecosystems, this thesis raises the question of the adjustment of FA composition, physiological and behavioral performance in fish. To investigate this question, this thesis aimed to combine observations in situ and experiments, and to use wild-caught sea bass juveniles to:

- Characterize the FA composition of wild sea bass juveniles, investigating the effect of ontogenetic stages on that composition, and comparing two different French habitats of sea bass juveniles: The Seine and Loire estuaries.
- Characterize the modulation of LC n-3 PUFA biosynthetic pathways investigating them (i) in wild juveniles, from different ontogenetic stages and from two different habitats (Loire and Seine estuaries), and (ii) in wild-caught individuals experimentally fed a dietary LC n-3 deficiency during several months.

- Evaluate experimentally the consequence of a LC n-3 PUFA deficiency on physiological and behavioral performances of wild-caught sea bass juveniles.
- Develop and to validate a non-lethal method to study the FA composition of individuals over time.

Reduction in dietary DHA production in marine food webs is believed to be more pronounced than for other LC n-3 PUFAs (Hixson and Arts, 2016). DHA is considered the most important FA for visual and cognitive functions in vertebrates, including fish (Pilecky et al. 2021). Thus, we focused on the effect of a dietary deficiency in DHA specifically on key physiological and behavioral performances of fish. We thus focused on growth, metabolic rates and swimming performance, as well as on the ability of sea bass juveniles to escape from a predator.

This manuscript is constituted by four chapters:

In **chapter 1**, we aimed to evaluate the influence of ontogenetic stage and habitat (estuary) on the FA composition and biosynthetic pathways in wild juvenile European sea bass. To do so, we conducted an *in-situ* analysis of the LC PUFA composition in three major organs (liver, muscle and brain) and investigated the biosynthesis abilities at transcript expression level in the liver of fish originating from the Seine and Loire estuaries.

The hypotheses of **chapter 1** were:

Hypothesis n°1: Ontogenetic stage influences FA composition and biosynthesis pathways because of 1) FA requirements varies according to the ages and 2) different trophic preferences

Hypothesis n°2: Estuary of origin influences FA composition because of the differences in primary production influencing the FA composition of the preys of European sea bass

In **chapter 2**, the aim was to conduct experiments to assess the physiological responses of wild-caught European sea bass when fed a diet limited in DHA. We investigated the effects on growth, aerobic swimming ability (U_{crit}) and metabolic rate. We also studied the impact of the DHA limitation on the expression of transcript coding for enzymes involved in FA biosynthesis.

The hypotheses of **chapter 2** were:

Hypothesis n°1: The dietary DHA limitation decreases the proportion of DHA in the tissue and therefore stimulates the biosynthesis of LC PUFA at the molecular level.

Hypothesis n°2: The dietary DHA limitation reduces growth rates because of the metabolic costs of the biosynthesis and its importance for growth.

Hypothesis n°3: Swimming performance and metabolic rates are influenced by dietary FA.

In **chapter 3**, we aimed to experimentally evaluate the effects of DHA dietary limitation on predator-prey relationships, studying the ability of wild European sea bass to escape from a predator.

The hypothesis of **chapter 3** was:

Hypothesis n°1: The dietary DHA limitation negatively affects the ability of wild European sea bass to escape a predator.

In **Chapter 4**, we aimed to develop and apply a non-lethal method for studying FA composition over time.

The hypotheses of **chapter 4** were:

Hypothesis n°1: The FA composition of biopsied white muscle is comparable to that of a conventional white muscle sample obtained by lethal method.

Hypothesis n°2: The FA composition results obtained from biopsies are repeatable.

Hypothesis n°3: Using the biopsy method, we can monitor dietary changes in FA composition at an individual level.

General Material and Methods

1. *In-situ study: Samplings in Seine and Loire estuaries*

To achieve the objectives of the first chapter of this thesis, focused on a field study, in-situ samplings were carried out during the NOURDEM campaign supported by Ifremer (<https://nourdem.ifremer.fr/>). This survey performs annual trawling campaigns in the estuaries of the Seine, Loire and Gironde in order to produce abundance indices for the species present in these nurseries and to assess the quality of the estuaries as nurseries. The ADJUST project, in which my PhD is included, obtained European sea bass juvenile in the estuaries of the Seine and Loire in 2019 and 2021.

1.1. Fish sampling

All the information concerning the sampling equipment used in the NOURDEM campaign can be consulted in the final report (Le Goff et al. 2022). In brief, a specific otter trawl of the Large Vertical Opening type was specifically made for this project. Trawl hauls lasted on average 15 minutes at an average speed of 3.5 knots. During the samplings, water temperature and salinity were continuously monitored.

Fish were sampled at different ontogenetic stages (ages) during the day by Ifremer's Laboratoire Biologie Halieutique team. Individuals born within the year (known as group 0 or G0) are generally found in the upstream, shallow, low salinity parts (Le Goff et al. 2017). Conversely, older juveniles colonize deeper waters further downstream in the estuary until they leave the nursery after the end of group 3 (G3, born in year $n-3$). This distinction is made on the basis of a size criterion established by an expert reference based on previous observations and growth curves (Le Goff et al. 2022). For European sea bass, this reference system is based on the following observed sizes: G0 = 0-11cm; G1 = 11-21 cm; G2 = 21-27 cm; G3 = 27+ cm. Samplings in the Loire took place on the 09, 10 and 11 of July 2019 from the port of Saint-Nazaire. Samplings in the Seine took place on the 07, 08 and 09 of August 2019 from the port of Honfleur. On both estuaries, all strata of the estuary were sampled (**Figure 8**) in order to maximise the capture of individuals of all ontogenetic stages (i.e. G1, G2 and G3). Too few G0 individuals were caught to study this group. The number of individuals caught, along with their stage and location, is detailed in **Table 1**. For the Chapter 1 of this thesis, 73 individuals were analysed.

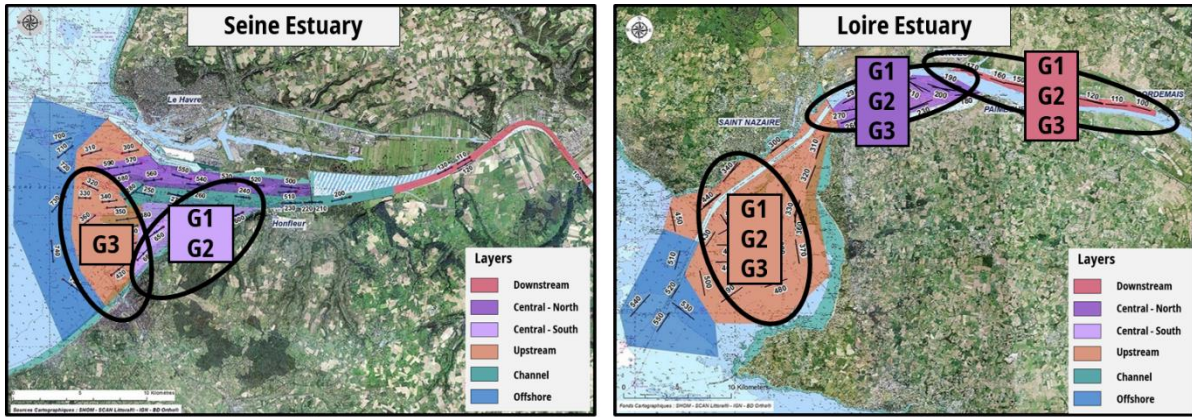


Figure 8: Sampling zones in estuaries of Seine and Loire in 2019. The coloured ellipses represent the different layers of the estuaries. Orange ellipses represent the downstream of the estuary, purple and pink ellipses represent the centre of the estuary and red ellipses represent the upstream of the estuary. The different life stages (G1, G2, and G3) sampled at these locations are indicated in the ellipses (Le Goff et al., 2022).

Table 1: Summary of the number of fish sampled during the NOURDEM campaign in 2019 in Loire and Seine estuaries.

Ontogenetic stages	Loire	Seine
G1	28 (18)	20 (12)
G2	22 (11)	21 (10)
G3	19 (12)	23 (12)
Total	69 (41)	62 (32)

1.2. Data collection

Caught individuals were kept in an on-board oxygenated 400 L tank containing the seawater in which they were caught. Once the boat went back to the port, the fish were individually euthanised with iso Eugenol (>5 mg L⁻¹). They were weighed and measured, and dissected on ice on board. About 150 mg of muscle, without skin, was removed from the left side, just behind the head, the liver was removed in its entirety, without the gallbladder, and the brain

was removed in its entirety. These samples were immediately flash-frozen in liquid nitrogen for later fatty acid (brain, muscle and liver) and molecular (liver) analysis. A small piece of muscle from the left side, just behind the brain (~30 mg) was removed and immersed in RNA Stabilization Reagent (RNAlater, Qiagen, Hilden, Germany) to be preserved for further molecular analysis (not analysed during the thesis). The scales were sampled under the pectoral fin and immersed in distilled water for further age checking. Otoliths were also sampled and preserved in ethanol (90°) for further micro chemical analysis (not presented in my thesis). Fin sample was also collected and preserved in ethanol (70°) for further genetic analysis (not presented in my thesis).

1.3. Age verification

Once back to the lab, the age of the sampled fish, classified into G1, G2 and G3 groups, was checked using scales. The age of an individual can be determined by studying the scales or otoliths and counting the growth streaks. For European sea bass, only the scales can be studied because sea bass otoliths become too opaque ([Figure 9](#)) after only a few months of life (Stéphane Martin, personal communication).

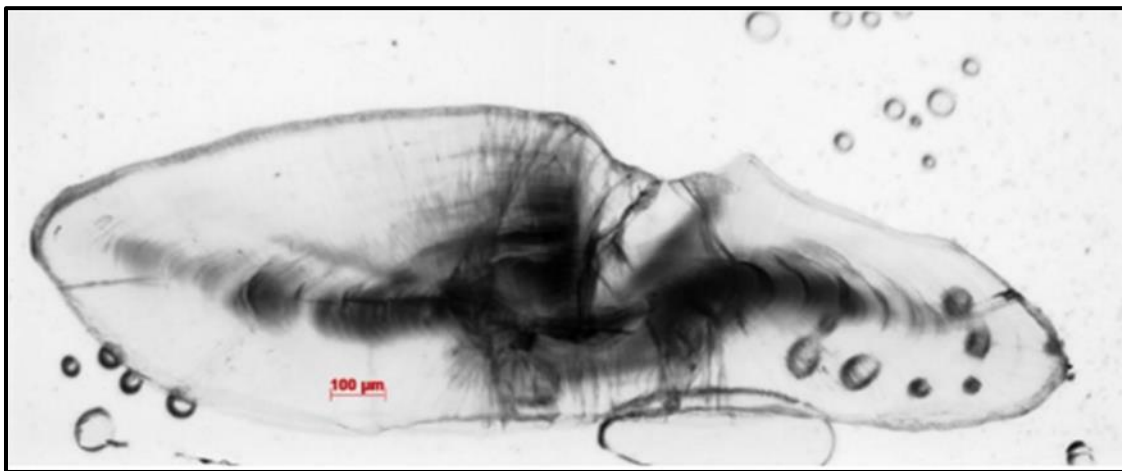


Figure 9: Photograph of a whole European sea bass otolith in cross-section at 0.8x magnification (Zeiss Stereo Lumar V12, Aurélie Libeau)

The age of an animal can be determined by reading the annuli of the scales under binocular loupe ([Figure 10](#), lines formed at the start of a growth season). The method for reading sea bass scales is described in detail in Fritsch (2005). For each fish, two different manipulators read at least two scales. The results of each manipulator were then compared to avoid bias.

In this manuscript and in the scientific article linked to this chapter, the term "Gx" is used after confirming the age of the individuals by reading the scales.

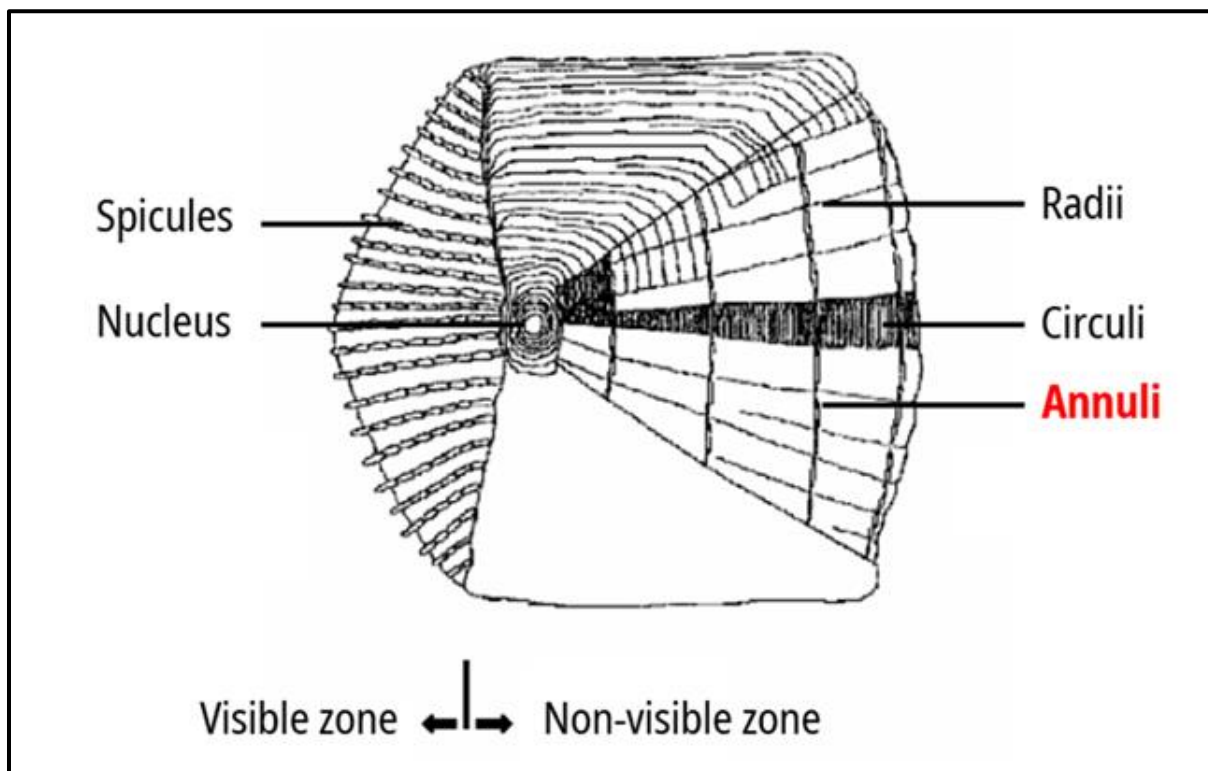


Figure 10: Schematic representation of the different parts of a scale of European sea bass (Adapted from Pickett and Pawson, 1994).

2. Experimental assessment of a dietary DHA deficiency on sea bass physiological and behavioural performances

This experimental part has been submitted and validated by the ethics committee (DAP30512_ 2021031811505740). This methods section corresponds to the Chapter 2 and Chapter 3 that studied the physiological and behavioral performances, respectively. The chronology of the whole experiment is presented in [Figure 12](#).

2.1. Fish capture and transportation

Approximately 150 fish of G1 ontogenetic stage were sampled during the NOURDEM campaign in 2021 (03 of August 2021) in the Seine estuary, and were carefully transferred, with a net, to a 400 L transport tank in a truck. This transport tank contained the water in

which fish were sampled that was oxygenated with a bubbler connected to an oxygen bottle to maintain optimum oxygen levels above 150% of air saturation. Oxygen and temperature were measured in the tank with a probe (OXI 3205, Dutscher, Germany) every 20 minutes during the first hour of transport, then every hour until arrival at the laboratory. Once the fish had been transferred to the transport tank (**Figure 11**), they were lightly sedated with tricaine (tricaine methanesulfonate, MS-222; 30 mg.L⁻¹) to minimise stress. On arrival at the laboratory, the animals were transferred by dip net, without direct contact with the manipulator, to a large 2000 L tank, in quarantine. The tank water was mixed with the transport water to limit any sudden changes in water parameters. The water was then progressively renewed using the open circuit of the experimental structures. Low mortality (~20%) was observed during the first days of the quarantine. Fish were treated with antibiotics (Oxytetracycline 200 ppm + Formol 30%, 200 ppm for 1h) to fight against a *Flexibacter maritimus* infection after the stress of transport.



Figure 11: Transport tank to bring the fish back to the laboratory

2.2. Chronology of the experiment and conditioning

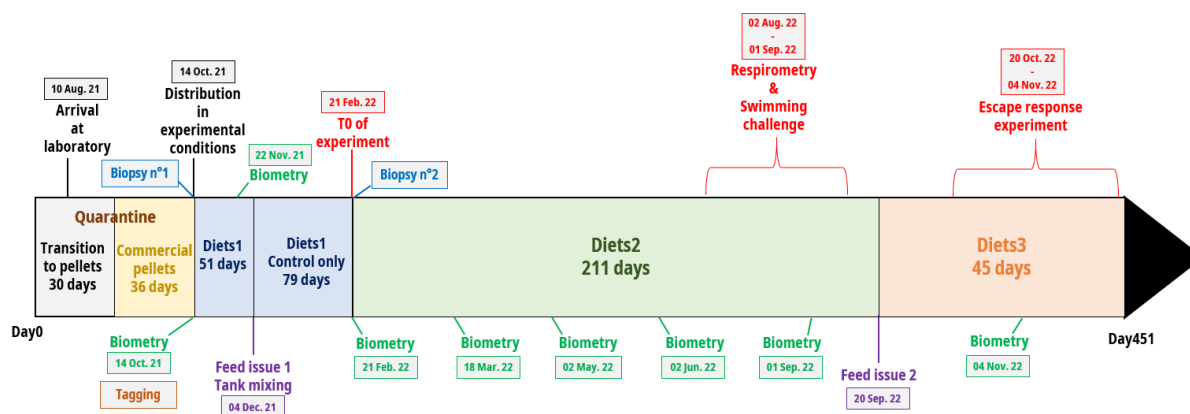


Figure 12: Schematic representation of the chronology of the experiment.

Quarantine

The mortality rate during the quarantine was estimated to be 30%. This number is an approximation because we could not count the exact number of fish in the tank after the transportation and realising a counting manipulation right after their arrival in the lab was considered too risky and stressful for the animals. During the quarantine, fish underwent a change from their natural diet to a pelleted diet by making a smooth transition. During the first week, the animals were fed a mixture of 75% defrosted seafood and 25% commercial pellets (NeoStart, Le Gouessant, Lamballe, France). The proportion of pellets was increased to 50% during the second week of quarantine, to 75% during the third week, and to 100% during the fourth week. The minimum quarantine period is 30 days as required by regulations, but the fish were kept in quarantine for a total of 70 days to allow them to fully recover from capture (observation of swimming and behaviour), transport, and acclimation to the experimental conditions.

Experimental conditioning, Diets1, 14 Oct. 21 – 21 Feb. 21

After 70 days of quarantine (i.e. on the 14 of October 2021), all fish ($n=101$) were anaesthetised (MS-222, 0.1 g.L^{-1}), weighed ($73.6 \pm 2.4\text{g}$), measured ($18.95 \pm 0.2 \text{ cm}$) and electronically identified using individual pit-tag (2 mm; M120; biolog-id, Réseautomatique, Bernay, France) (Figure 14). Fish underwent a subcutaneous white muscle biopsy using a biopsy punch (2 mm LCH-PUK-20, Kai Medical, Solingen, Germany) to track their fatty acid composition at the beginning of the experiment without killing them. Fish were then randomly

distributed into six 500L-experimental oxygenated tanks (n=16 or 17 fish per tank) supplied with seawater (350 L h^{-1}) from the bay of Brest via an open circuit allowing the water to be renewed. The water was filtered using a sand filter and sterilised using a UV lamp. The salinity of the water was 35 and the oxygen saturation level was never below 90%. The water temperature fluctuated according to the seasonal rhythm of the bay of Brest (**Figure 13**). The photoperiod was set at 12 h day/12 h night.

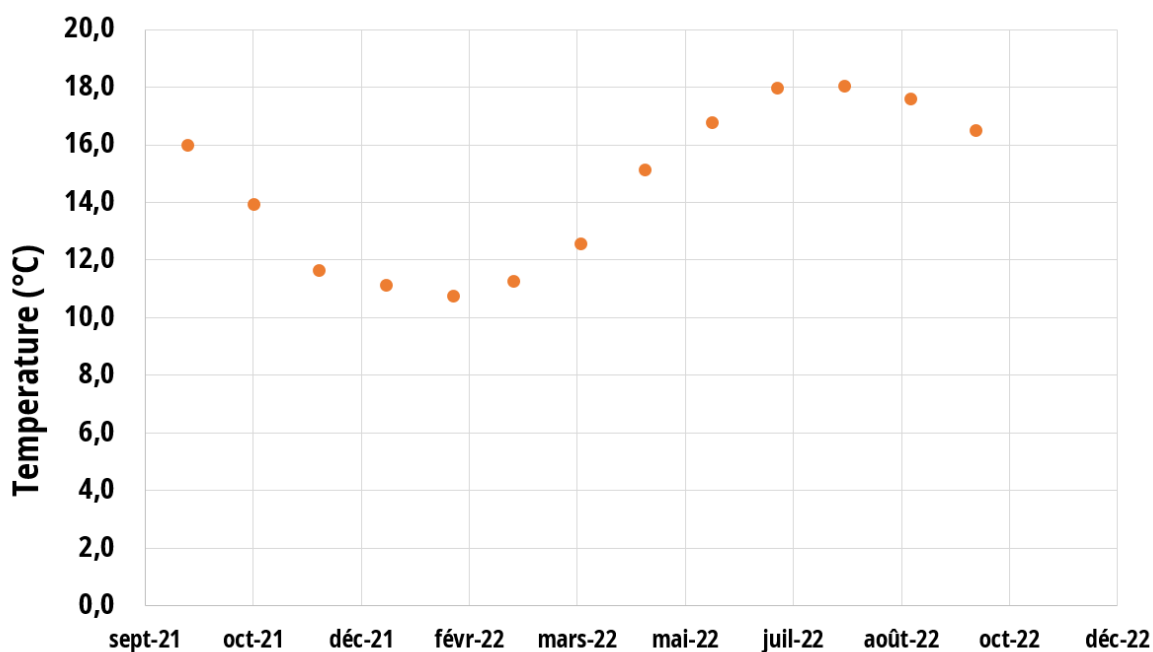


Figure 13: Temperature of the seawater in the experimental tanks during the whole experiment

Fish were fed either a Control (n=3 tanks, i.e. 51 fish in total) or a DHA Depleted diet (n=3 tanks, i.e. 50 fish in total; **Figure 15A**), named Diets1 (**Table 2**). Both diets were isolipidic and isoproteic. The Control diet contains standard levels of DHA for European sea bass (Skalli and Robin 2004), while in the Depleted diet, fish oil was replaced by a microalgae oil (*Nannochloropsis* spp.) provided by Polaris (Quimper, France), which was supposed to contain high levels of EPA and be deprived in DHA (**Table 2**). However, some DHA was still present in the depleted diet, coming from the fishmeal used to reach the protein and amino acids requirements. The diet containing fish oil is called “Control diet” and the diet containing Polaris oil is called “Depleted diet”. These diets were manufactured by INRAE (Donzacq, France). During the first month of the experiment, in order to control food intake, the fish were fed twice a day by hand. The amount of food given depended on the water temperature

and was between 0.6 and 2% of the total biomass of the tank. After the first month, the animals were fed using an automatic feeder. The accumulation of faeces or uneaten food was evacuated each day using a purge, and daily cleaning of the tanks limited the accumulation of ammonia and nitrate-nitrite.

Table 2: Fatty acid composition of the Diets1

Ingredients (g.100g ⁻¹)	Control Diet1	Depleted Diet1
Fish Meal	24	24
Casein	37	37
Canola oil	2	3
Fish oil	8	0
Polaris oil	0	7
Starch	15	15
Vitamins	4	4
Minerals	2	2
Cellulose	5	5
Betaine	3	3
FA (% of total FA)	Control - Diets1	Depleted - Diets1
14:0	5.9 ± 0.0	0.8 ± 0.1
15:0	0.4 ± 0.0	0.1 ± 0.0
16:0	15.1 ± 0.1	3.6 ± 0.2
17:0	0.4 ± 0.0	0.1 ± 0.0

General Material and Methods

18:0	3.6 ± 0.0	2.5 ± 0.0
20:0	0.3 ± 0.0	0.8 ± 0.0
22:0	0.2 ± 0.0	0.2 ± 0.0
ΣSFA	25.9 ± 0.2	8.1 ± 0.3
16:1n-7	6.1 ± 0.0	0.8 ± 0.1
16:1n-9	0.2 ± 0.0	ND
18:1n-7	3.0 ± 0.0	1.8 ± 0.0
18:1n-9	17.8 ± 0.2	14.3 ± 0.1
20:1n-9	1.7 ± 0.1	3.5 ± 0.0
ΣMUFA	32.6 ± 0.1	23.4 ± 0.3
18:2n-6	4.9 ± 0.1	4.5 ± 0.1
18:3n-3	2.2 ± 0.0	2.0 ± 0.0
20:4n-6	0.9 ± 0.0	2.9 ± 0.1
20:5n-3	14.4 ± 0.2	45.2 ± 0.7
22:5n-3	1.9 ± 0.1	0.9 ± 0.0
22:6n-3	7.7 ± 0.1	6.0 ± 0.1
ΣPUFA	40.5 ± 0.3	67.6 ± 0.7
ΣLC PUFA	25.8 ± 0.1	55.6 ± 0.6
Σn-6	6.7 ± 0.1	8.4 ± 0.1
Σn-3	26.9 ± 0.1	56.0 ± 0.6

General Material and Methods

EPA/DHA	1.9 ± 0.1	7.5 ± 0.3
ARA/EPA	0.07 ± 0.0	0.06 ± 0.0



Figure 14: Picture of the tagging of the fish (14 Oct. 2021)

Feed issue 1 and tank mixing (04th of December 2021)

51 days after fish were fed the experimental diets¹ (i.e. on 04 of December 2021) we realised that the diet supposed to be DHA-depleted was in fact not really so, due to the real DHA content of the Polaris oil being higher than the theoretical content announced. The decision was made to mix half of the individual from the “depleted” condition with controls (**Figure 15B**) to start-over in order to smooth the FA composition of the fish. After the mixing, all tanks were fed with the Control diet until manufacturing of new pellets (i.e. 60 days after the mixing).

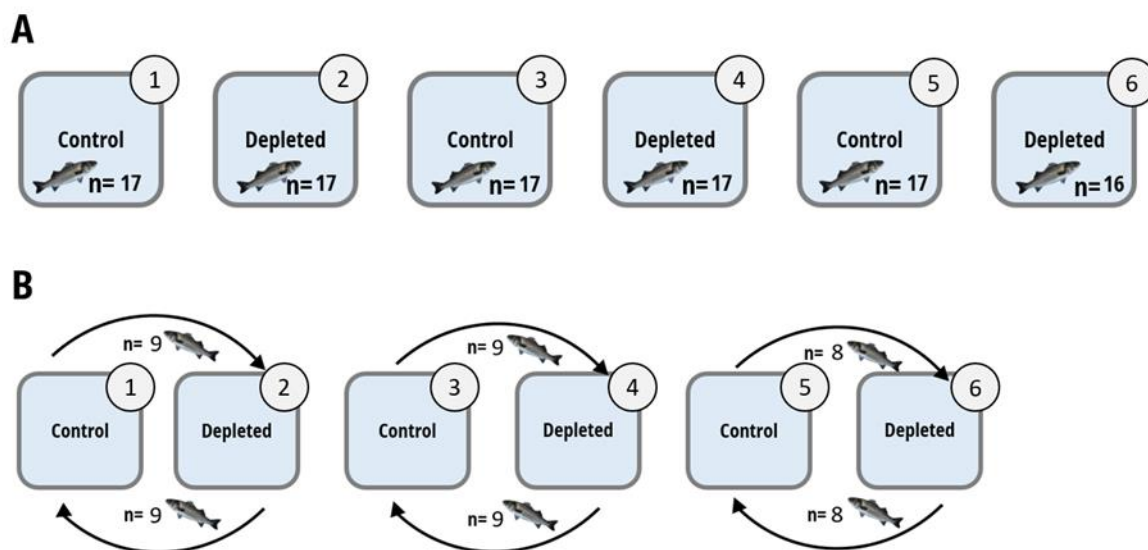


Figure 15: Schematic representation of the experimental design and distribution of individuals among conditions (A). (B) represents the exchange between individuals in the control condition and the deficient condition after being fed an experimental food containing excessively high levels of DHA. Numbers in circles indicate the tank number.

Experimental conditioning (Diets2; 21st February 2022 to 1st September 2022)

When the new pellets were received (Diets2), they were thoroughly tested for FA composition (according to the protocol described section 3.1) (**Table 3**). The casein present in the Diet1 (**Table 2**) was replaced with wheat gluten to improve the palatability of the feed for fish. The detailed formulation of the Diets2 is presented in **Table 3**. Another white muscle biopsy was performed to track the FA composition on 02nd of February 2022. Starting on the 21 of February 2022, the individuals started a minimum of 5 months dietary conditioning in their respective conditions.

General Material and Methods

Table 3: Formulation, chemical composition and fatty acids composition of the experimental diets (Diets2). Data are presented as mean \pm sd (n=3). ARA: Arachidonic acid; DHA: Docosaheaxaenoic 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 section 3.1 of the material and methods section for fatty acid analysis protocol.

Ingredients (g.100g ⁻¹)	Control Diet2	Depleted Diet2
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
Chemical composition (g.100g ⁻¹ \pm SD) - Analysed by UpScience		
Proteins	59.2 \pm 1.8	59.5 \pm 1.8
Lipids	13.8 \pm 1.0	13.1 \pm 1.0
Nitrogen	9.5 \pm 0.3	9.5 \pm 0.3
FA composition (% of total FA)		
14:0	4.9 \pm 0.5	2.2 \pm 0.5
15:0	0.3 \pm 0.5	0.2 \pm 0.5
16:0	15.1 \pm 1.2	12.8 \pm 1.0
17:0	0.1 \pm 0.5	0.1 \pm 0.5
18:0	3.2 \pm 0.5	1.2 \pm 0.5

General Material and Methods

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
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

Feed issue (20 Sep. 2022)

In August 2022, i.e. after a minimum 5 months of fish conditioning with Diets2, the quantity of the Depleted diet was low and not enough to extend the experiment to the end. Then, the same diet was ordered to INRAE. After receiving and testing this feed, the FA profiles turned

out to be very different from the feed used during the 5-month experiment. Given time required to manufacture a new diet at INRAE at this period, we decided to manufacture the both Control and Depleted diets in our own laboratory (Diets3; **Table 4**), with the same formulation as that of Diets2 (**Table 3**). We obtained the raw materials (Fishmeal, Fish oil, Wheat gluten, Canola oil, Starch, Vitamins, Minerals, and Cellulose) from Le Gouessant manufacture (Lamballe, France). The FA profiles of Diets3 were similar to that of Diets2, but, because of the different sources of raw materials and the different manufacturing process from that used to produce the Diets2, the DHA proportions were not as low as in Diets2 (**Table 4**). The detailed manufacturing protocol of Diets3 is available in Appendix 1. The fish were then fed with Diets3 until the end of the experiment, i.e. 4th of November 2022, i.e. during 45 days (**Figure 12**).

Table 4: Comparison of the fatty acid composition of the Diets2 (n=3) and Diets3 (n=3) (Diet2 and Diet3)

FA (% of total FA)	Control Diet2	Control Diet3	Depleted Diet2	Depleted Diet3
14:0	4.9 ± 0.5	2.66 ± 0.0	2.2 ± 0.5	2.0 ± 0.0
15:0	0.3 ± 0.5	0.3 ± 0.0	0.2 ± 0.5	0.2 ± 0.0
16:0	15.2 ± 1.2	15.4 ± 0.1	12.8 ± 1.0	13.6 ± 0.2
17:0	0.1 ± 0.5	0.3 ± 0.0	0.1 ± 0.5	0.2 ± 0.0
18:0	3.2 ± 0.5	2.8 ± 0.0	1.2 ± 0.5	1.3 ± 0.0
20:0	0.4 ± 0.5	0.4 ± 0.0	0.4 ± 0.5	0.3 ± 0.0
22:0	0.2 ± 0.5	0.2 ± 0.0	0.2 ± 0.5	0.2 ± 0.0
∑ SFA	24.8 ± 1.0	21.9 ± 0.1	17.9 ± 1.5	17.8 ± 0.2
16:1n-7	4.8 ± 0.5	4.1 ± 0.0	9.4 ± 0.8	8.5 ± 0.1
16:1n-9	0.1 ± 0.5	0.3 ± 0.0	0.5 ± 0.5	0.6 ± 0.0
18:1n-7	2.8 ± 0.5	2.8 ± 0.0	2.0 ± 0.5	2.0 ± 0.0
18:1n-9	22.7 ± 1.8	24.7 ± 0.2	30.0 ± 2.4	26.5 ± 0.3
20:1n-9	1.5 ± 0.5	1.2 ± 0.0	0.6 ± 0.5	0.7 ± 0.0
∑ MUFA	35.7 ± 2.3	35.0 ± 0.1	43.8 ± 2.6	39.1 ± 0.3
18:2n-6	12.6 ± 1.0	17.9 ± 0.2	17.0 ± 1.4	18.6 ± 0.3
18:3n-3	2.9 ± 0.5	3.9 ± 0.0	5.1 ± 0.5	4.5 ± 0.0
20:4n-6	0.8 ± 0.5	0.7 ± 0.0	1.5 ± 0.5	1.5 ± 0.0
20:5n-3	10.7 ± 0.9	6.5 ± 0.0	13.2 ± 1.1	12.2 ± 0.1
22:5n-3	1.5 ± 0.5	1.0 ± 0.1	1.2 ± 0.5	0.2 ± 0.0
22:6n-3	5.3 ± 0.5	7.6 ± 0.1	0.5 ± 0.5	2.5 ± 0.0
∑ PUFA	39.6 ± 2.5	42.5 ± 0.2	39.5 ± 2.4	42.0 ± 0.4
∑ LC PUFA	18.6 ± 1.2	21.5 ± 0.2	15.7 ± 1.0	16.6 ± 0.1
∑ n-3	22.7 ± 0.5	19.4 ± 0.2	19.9 ± 1.0	20.2 ± 0.1

$\Sigma n-6$	14.0 ± 1.1	19.4 ± 0.2	19.3 ± 1.2	20.8 ± 0.2
EPA/DHA	2.0	0.9	26.4	4.8
ARA/EPA	0.07	0.11	0.11	0.12

2.3. Measurements of growth

All along the experiment, fish growth and survival was monitored during biometry (**Figure 12**). For each biometry, all the fish were anaesthetised (MS-222, 0.2 g.L⁻¹), electronically identified, weighted and measured. The following indicators were then calculated:

- The survival rate :

$$Survival = 100 \times \left(\frac{\text{Number of individuals alive}}{\text{Number of total individuals}} \right)$$

- The Thermal Growth Coefficient (TGC, g degree days⁻¹) :

$$TGC = 1000 \times \left(\frac{W_{final}^{1/3} - W_{initial}^{1/3}}{T \times D} \right)$$

Where W_{final} is the weight at the end of the experiment, $W_{initial}$ is the weight at the beginning of the experiment, T is the average temperature during the experiment and D is the number of days between the disposal into experimental conditions and the experiments.

- The Fulton's condition factor (K, arbitrary unit):

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

- The Le Cren relative condition factor (K_n , arbitrary unit):

$$K_n = \frac{W}{aL^b}$$

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.

2.4. Measurements of metabolic rate and swimming performance

After being conditioned for 162-192 days with the Depleted or the Control diet, a swimming challenge experiment, based on an U_{crit} protocol, was carried out on individuals from both conditions, using a swimming tunnel (Loligo® Systems, **Figure 16**). The U_{crit} protocol is a method used to assess the maximal aerobic and swimming performance of fish. The swimming challenge began on 02 August 2022 and ended on 01 September 2022.

2.4.1. Animals

A total of 18 fish for the control condition (Weight = 116.0 ± 6.4 g; Total Length = 21.2 ± 0.3 cm) and 18 fish for the deficient condition ($W = 107.5 \pm 5.0$; $TL = 21.1 \pm 0.4$ cm) were tested. One fish from each condition was challenged each day ($n=2$ fish per day).

2.4.2. Swimming tunnel

Two swimming tunnels (Loligo® Systems, Tjele, Denmark, **Figure 16A**) were used in parallel each day. They were loaned to us by LIENSS (La Rochelle, France) in collaboration with Christel Lefrancois. For each tunnel, the respirometer (10 L) consisted of a swim chamber (40 L x 10 H x 10 W cm; N°1, **Figure 16**) and a hydraulic system creating a laminar flow thanks to a honeycomb structure (N°2, **Figure 16**) placed upstream of the swim chamber. The current was generated using an external motor (N°3, **Figure 16**) turning a propeller (N°4, **Figure 16**). For each tunnel, the motor was calibrated using a current metre before starting the experiments and a flush pump (N°5, **Figure 16**) with the water going out of the swim chamber with a hose

(N°6, **Figure 16**) was used to maintain an optimal oxygen level in the swimming chamber using the following cycle : 20 minutes of measurement followed by 5 minutes of oxygen restoration (while measuring the oxygen) provided by the flush pump. A black tarpaulin was placed on the outside of the swimming chamber (**Figure 16B**) to limit visual contact with the animal and thus limit its stress. As this cover blocked direct vision of the fish, two webcams (Logitech C920s PRO) were used to visualise the fish throughout the experiment.

The tunnels were filled with filtered seawater from the bay of Brest and sterilized with an UV lamp. The temperature of the water was between 19° and 20°C. The photoperiod was set on 12h day / 12 h night. The salinity was checked before putting any fish in the tunnel to ensure that the freshwater used to clean the tunnels was rinsed properly.

Oxygen level (relative to fish oxygen consumption) and temperature were continuously monitored in the swim chamber using fibre-optic probes (FireSting O₂, PyroScience, Germany, N°7; **Figure 16**) (one data point per second). These probes were calibrated before each experiment and the data was monitored using PyroScience Workbench software (V1.3.9.2140).

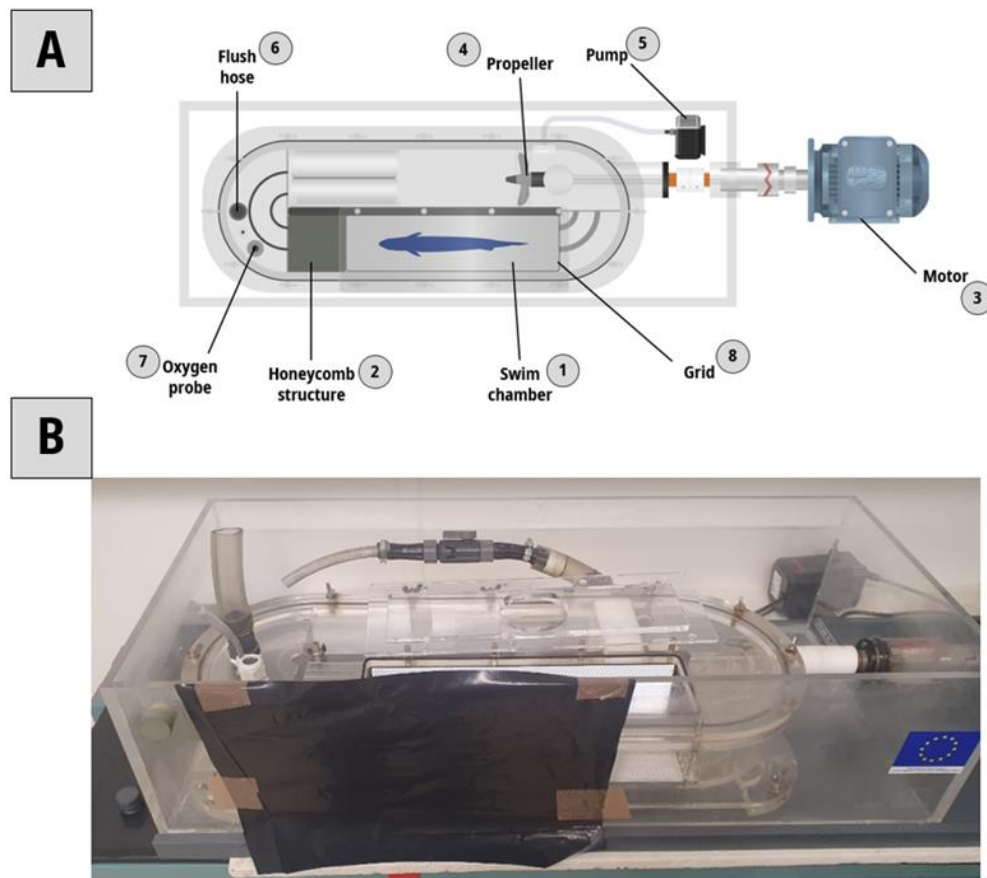


Figure 16: Schematic representation of the swimming Tunnel (A; modified from Loligo® Systems) and picture of the swimming tunnel with the black tarpaulin to reduce stress (B).

2.4.3. Swimming challenge

Before the challenge

Before the experiment, fish were caught in their experimental tank (1 fish per experimental condition) using a net, and isolated from the rest of the individuals in a 1000 L tank to be fasted for 48 hours to avoid any effect of digestion on the further measure of the standard metabolic rate. The next day, two other individuals were caught from two different experimental tanks and placed in the same isolation tank as the two first fish. The day before the experiment, i.e. after 48 hours of fasting, the first two fish were caught with a net, placed in a plastic bag to avoid the contact of the gills with air that could affect metabolism (Claireaux et al. 2005), and placed in a bucket containing anaesthetic solution (MS-222, 0.1 g L⁻¹). Once anaesthetised, the fish were replaced in the plastic bag, electronically identified through the

bag, approximately measured in the bag in order to further set the swimming speeds in body length per second (BL s^{-1}), and placed randomly into the swimming tunnels (1 fish per swimming tunnel).

After the fish recovered from anaesthesia in the swimming tunnel, the speed of the current was set to 0.5 BL s^{-1} for 10 minutes. The fish was then trained to swim against the current for an additional 10 minutes, with the speed being increased slowly and progressively. Following this training, the swimming speed was reset to 0.5 BL s^{-1} , and the fish was left in the tunnel overnight. During this period, its oxygen consumption was measured in cycles consisting of 20 minutes of measurement followed by 5 minutes of oxygen restoration provided by the flush pump.

The swimming challenge

The next morning, the animals were subjected to a step protocol (Brett, 1964, **Figure 17**), also known as U_{crit} test. It was carried out on each fish to obtain the critical swimming speed of an animal called U_{crit} corresponding to the aerobic maximal swimming speed. The speed was increased over 5 min, by 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. Each swimming step lasted 20 min. The associated oxygen consumption was measured concurrently for durations of 20 minutes, with recirculation occurring during the 5-minute increments of speed increase. Oxygen saturation never dropped under 75%. Fish were considered exhausted when they failed to swim off the grid (**N°8**, **Figure 16**) for 15 s after light stimulation.

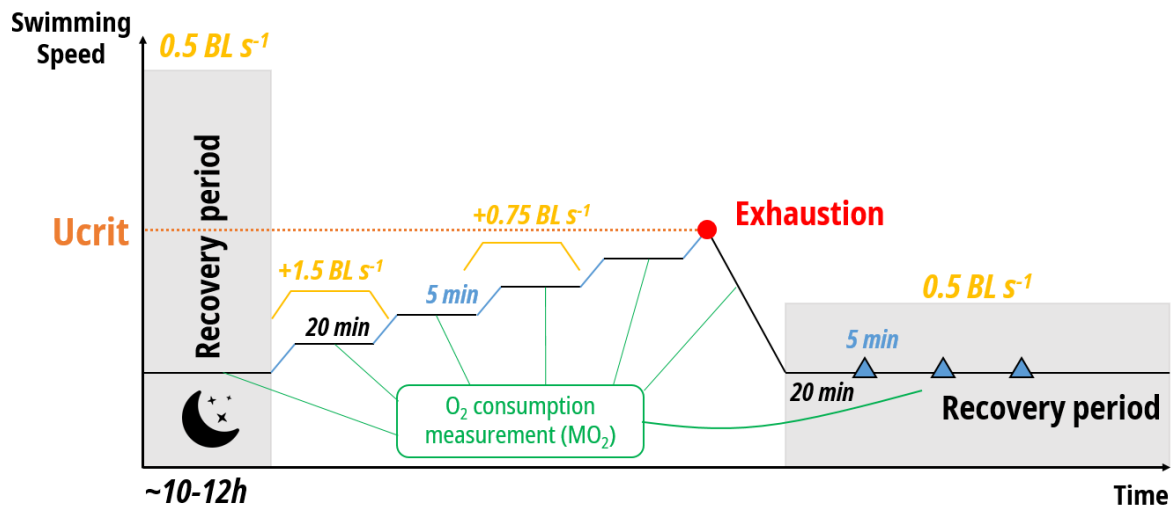


Figure 17: Schematic representation of the critical swimming protocol (Brett, 1964). The y axis represents the time and the x axis represents the swimming speed. Oxygen consumption measurements (MO_2) are indicated with the green lines. Swimming speed during the recovery periods (grey zones) was 0.5 BL s^{-1} . After the first recovery period (overnight), swimming speed was increased two times by 1.5 BL s^{-1} and then by 0.75 BL s^{-1} every 20 min until the fish reached exhaustion. These 20 min measurements were separated by periods of 5 min (blue lines and triangles) where the oxygen levels were brought to saturation using the flush pump.

After the challenge – recovery

When exhaustion was reached, the velocity of the current was immediately decreased to the recovery speed (0.5 BL s^{-1}), and fish oxygen consumption was measured for 1 hour, as previously described (*i.e.* 20 min measurement, 5 min flush; *i.e.* three measurements), to assess the fish recovery capacity in terms of excess post-oxygen consumption (EPOC). EPOC quantifies the energy and time needed to restore the tissue and cell storage of oxygen, the balance levels of metabolites such as lactate and glycogen and other metabolic functions such as ionic and osmotic balance (Lee et al. 2003).

2.4.4. Swimming performance, oxygen consumption and metabolic rates

Critical swimming speed

U_{crit} was measured using the following equation Brett (1964):

$$U_{crit} = U_{max} + \frac{t_1}{t} \times U_1$$

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

The water flow speed in the swim chamber is affected by the solid-blocking effect (SBE) when a fish occupies a portion of the cross-sectional area. As a result, we made adjustments to the swimming speeds using the formulas from Bell and Terhune (1970):

$$U_F = U_T(1 + \varepsilon_s)$$

where U_F is the corrected flow speed and U_T is the speed in the flume without a fish in the swimming section. The fractional error due to solid blocking (ε_s) was calculated for each individual fish as:

$$\varepsilon_s = \tau\lambda(A_O/A_T)^{3/2}$$

Oxygen consumption

To calculate the metabolic parameters, the oxygen consumption measured ($MO_{2\ meas}$) was first calculated following this equation:

$$M_{O_{2\ meas}} = \left[\left(\frac{\Delta[O_2]}{\Delta t} - \frac{\Delta O_2}{\Delta t_{bact}} \right) \right] \times \left(\frac{v}{m} \right)$$

$\Delta[O_2]/\Delta t$ is obtained by plotting $[O_2]$ versus time to adjust a linear regression (**Figure 18**) (Graphical Analysis 3.4, Beaverton, OR, USA). Only slopes with a coefficient of regression (R^2) above 0.90 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.

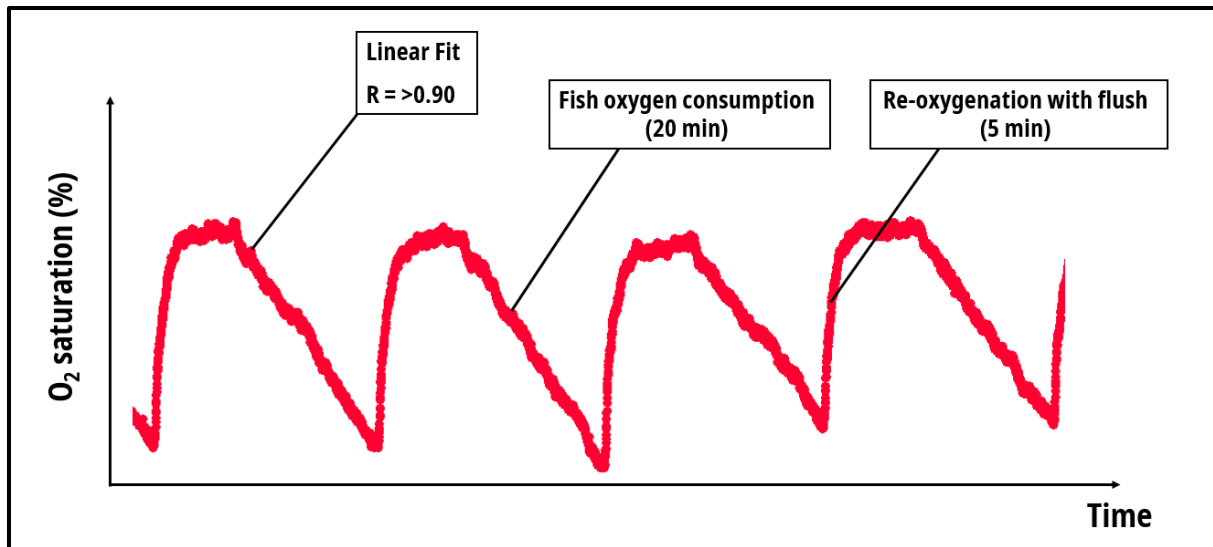


Figure 18: Schematic representation of the oxygen consumption data. O₂ saturation is represented as a function of time. The decreasing slope represents the diminution of oxygen in the swim chamber (i.e. oxygen consumption). The increasing slope represents an increase in oxygen in the swim chamber (i.e. re-oxygenation of the swim chamber with the flush). Oxygen consumption was measured with equation X and only slopes with a coefficient of regression above 0.90 were used.

As the animal weight influences the respiratory metabolism, MO₂ was standardised (MO_{2 cor}) with a standard fish mass of 100 g (Steffensen 1989), with the following equation:

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

Where MO_{2cor} is the oxygen consumption for a fish with a standard mass of 100 g (*m_{corrected}*), MO_{2 meas} is the calculated oxygen consumption and *m* is the fish weight (kg). *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).

Metabolic rates

Standard Metabolic rate (SMR) (mg O₂ kg⁻¹ h⁻¹) was estimated by extrapolating the following equation to a null swimming velocity (Brett 1964):

$$M_{O_2\ cor} = SMRb^U$$

Where SMR is the intercept, *b* is a constant and *U* is the swimming speed (BL s⁻¹).

Maximal Metabolic Rate (MMR; $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was estimated by taking the highest value of MO_2 during the swimming challenge (Norin and Clark 2016).

Aerobic scope (AS; $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was calculated with the following equation (Lefrançois and Claireaux 2003):

$$AS = MMR - SMR$$

Excess Post-Oxygen Consumption

EPOC ($\text{mg O}_2 \text{ kg}^{-1}$) 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).

Cost of transport

The net cost of transport at maximum exercise (NCOT in mg O₂ kg⁻¹ m⁻¹) was calculated as in Clark et al. (2011):

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

Where U_{crit} is converted from BL s⁻¹ into m min⁻¹.

After the month of experimentation in the swimming tunnel, the remaining animals were conditioned in the same way until 20 September 2022.

2.5. Evaluation of escape response

2.5.1. Protocole of the escape response test

After 30 days (20 Sep. 2022 - 20 Oct. 2022) of fish conditioning with Diets3, the impact of diet on fish escape response was measured on 36 fish (18 fish per dietary condition; $W_{Control} = 230.1 \pm 16.3$ g; $TL_{Control} = 26.4 \pm 0.6$ cm; $W_{Depleted} = 244.1 \pm 24.9$ g; $TL_{Depleted} = 27.3 \pm 0.9$ cm) using an internal mechano-sensory stimulus (Lefrançois et al. 2005). Two fish, coming from the two different experimental conditions, were tested each day, thanks to our two experimental devices (**Figure 19**). Four additional fish from our experiment were tested but were not considered for analysis as they were used only to refine our experimental setup.

Before the test

Before the test, two fish (one fish per experimental condition) were caught in their respective experimental tank using a net, electronically identified, and isolated from the rest of the individuals in a 1000 L tank to be fasted for 48 hours. The next day, two other fish were caught from two other experimental tanks (one fish per experimental condition), electronically identified, and placed in the same isolation tank as the two first. The day of the experiment, i.e. after 48 hours of fasting, the two isolated fish were caught with a net, anaesthetized in a bucket (MS-222; 0.1 g L⁻¹), electronically identified with their pit-tag, and transported to the testing tanks (**Figure 19A**). The fish were acclimated to the testing tank during one hour to reduce their stress and allow them to explore their new environment. To avoid any differences linked to the chronobiology, all fish were tested in the morning (from 9:00 to 13:00).

The escape response test

The mechano-sensory stimulus consisted in a falcon tube filled with sand (**Figure 19**, A1; 43g) that was attached to an electro-magnet connected to a switch (**Figure 19**, A2). Turning the switch off allowed the drop of the stimulus, attached to a wire, to drop through a PVC tube inside the water without touching the bottom of the tank. This tube, painted in black, hides the stimulus so the fish did not notice it until it touched the water, imitating an avian predator in a turbid environment (Lefrançois et al. 2005). This allowed us to observe the exact moment of stimulation (*i.e* the video frame when the stimulus touched the water). Simultaneously to the triggering of the switch, a video recording the escape response was manually started using a high speed camera (AOS S-MOTION, 500 frames per second, AOS Technologies, Switzerland), and using the OAS Imaging Studio software (**Figure 19**, A3). To monitor the fish behaviour during all the experiment, a webcam (**Figure 19**, A4; Logitech C920s PRO) was placed inside the tank and connected to a laptop (**Figure 19B**).

The triggering of the switch required that:

- (i) The fish was positioned within the camera field of view not too close to the stimulus (**Figure 19B**)
- (ii) The speed of the fish must be null or very slow
- (iii) The surface water must be still and without waves

If after 13:00, these conditions were not fulfilled, the fish was taken out of the experimental tank without being tested again.

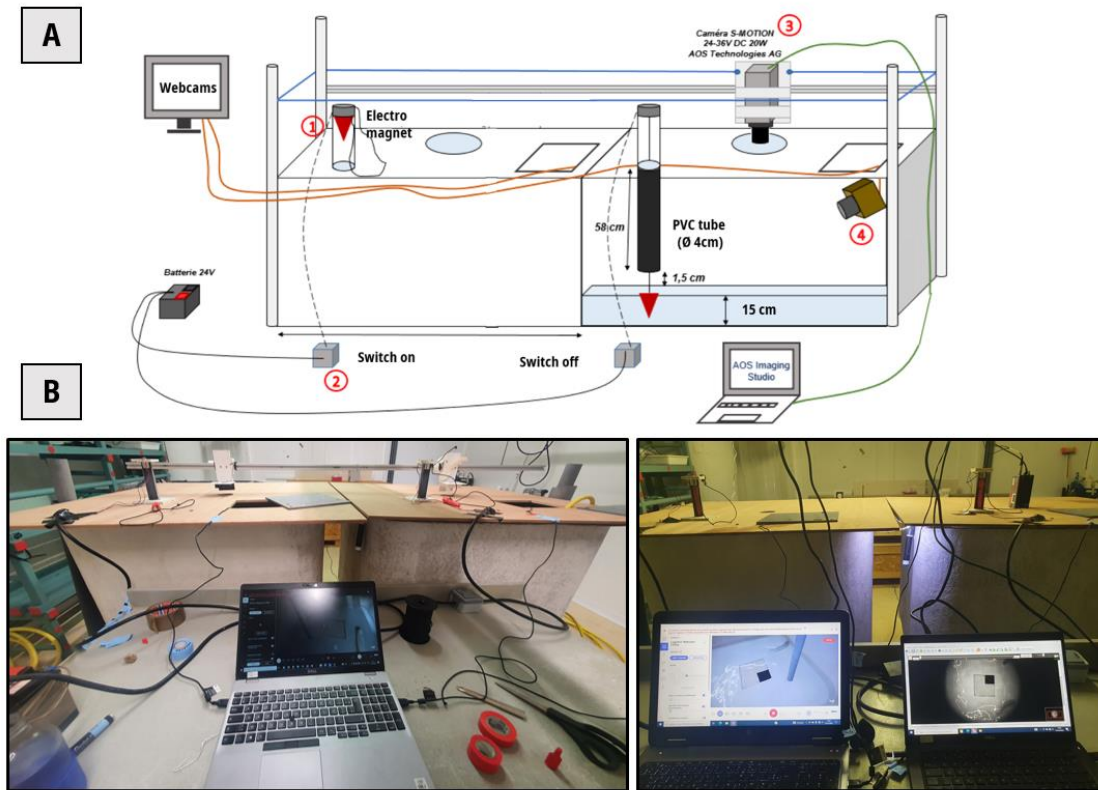


Figure 19: Schematic representation of the experimental setup (A, credit: Salomé Ducos Master 1, 2018) and pictures of the experimental setup (B).

After the test

After obtaining the video, the tested fish were caught with a net, and transferred in a bucket of seawater and euthanized (MS-222 concentration $>0.4 \text{ g L}^{-1}$). Then, the fish were individually weighed and measured. The muscle (on the left side, just behind the head), the liver (without gallbladder), the brain and the eyes were sampled and immediately frozen in liquid nitrogen for further fatty acid and molecular analysis. The brain and the liver were weighed to obtain the cerebro-somatic index (CSI) and hepatosomatic (HSI).

2.5.2. Video analysis and measured variables

Software

All of the videos were analysed with the software Kinovea (V0.9.5). The videos were encoded with the “Cinepak” codek in .avi format

Responsiveness

The responsiveness represents the proportion of escape response on the total stimulations (Domenici and Hale, 2019). Basically, it corresponds to answering yes or no to the question: “Did the fish escape after stimulation?”

Latency

The latency corresponds to the duration (in millisecond) between the moment when the stimulus is visible and the moment when the fish triggers an escape response (*i.e* the frame when a movement is visible).

Distance covered

The distance covered (in cm) corresponds to the distance between the centre of mass (CM) of the fish at the frame before the escape response was triggered and the CM of the fish 70 ms later (Domenici et al. 2007; Carvalho et al. 2022). The CM of the fish was determined to be at 38% of the length of the fish, starting from the tip of the head (Lefrançois and Domenici 2006)

Turning rate

The turning rate (degree second⁻¹) represents the angular velocity of the escape response (Domenici and Hale 2019). In other words, it is how fast a fish turns after triggering the escape response. It is the ratio between the turning angle of stage 1 and the duration of stage 1 (Domenici et al. 2007).

3. Tissue sampling

3.1. Justification of the tissue sampled

The liver plays a crucial role in fish, serving as a primary location for both the storage and biosynthesis of FA (Guil-Guerrero et al., 2011; Tocher, 2003). In lean fish species, such as sea bass, a significant portion of their FA can be found in the liver, which can contain lipid levels exceeding 50% (Guil-Guerrero et al. 2011). It is also an organ in which expression of genes involved in FA synthesis has been widely studied (Mourete et al. 2005; Mourete and Bell 2006; Geay et al. 2010a). Additionally, the liver is a recognized marker for assessing the nutritional and health status of fish (Segner and Juario 1986; Caballero et al. 1999, 2004). This makes the liver an ideal tissue for studying lipids and FA in fish.

The muscle constitutes about 50% of the fish's total mass (McKenzie 2011). Its primary function is to facilitate movement through muscle contraction, which in turn affects vital ecological activities like foraging, reproduction, migration, and overall the maintenance of the population (Sänger et al. 2001; McKenzie 2011). The predominant component of fish skeletal muscle is white muscle, which is the edible part constituting a vital source of protein and essential FA for humans (Mohanty et al. 2019; FAO 2020). Additionally, in lean fish, the muscle serves as a storage area for lipids (Venugopal and Shahidi 1996).

The brain tissue in vertebrates is known to retain LC n-3 PUFA, in its phospholipids. Among these LC n-3 PUFA, DHA plays a pivotal role in brain and neural functions (Tocher and Harvie 1988), see section “Behavioral performance” of introduction for further details). However, there is a debate regarding the influence of dietary FA on the FA composition of fish brains. Some researchers argue that a fish's diet can impact the lipid content of its brain (Pagliarani et al. 1986; Tocher and Harvie 1988), while others believe it remains stable regardless of diet in order to preserve cognition (Skalli et al. 2006). A recent study indicated that fish fed on a low DHA diet for an extended period (5 months) had reduced DHA content in their brain, compared to those fed on a standard DHA diet (Carvalho et al. 2022). Interestingly, this difference was not observed in these fish when they were fed these respective diets for only 2.5 months (Carvalho et al. 2022).

3.2. The subcutaneous white muscle biopsy

The decision to perform a subcutaneous white muscle biopsy was motivated by the will to track individually the FA composition over time during the experimental period. Usually, muscle sampling for fatty acid analysis on juvenile fish is lethal, then it does not allow us to monitor the FA composition over time, and reduce the number of individuals available for further analysis. We relied on a previous study on the same species to ensure that the biopsy procedure was safe for the animal and did not affect it negatively in terms of growth and immune response (Quéméneur et al. 2022). We then developed a method to apply the subcutaneous white muscle biopsy for FA analysis and it made the object of a publication in review for Fish Physiology and Biochemistry. This article is available in the chapter 4 of this manuscript and the following part will describe in detail the biopsy procedure (Figure 20) but will not discuss the results.

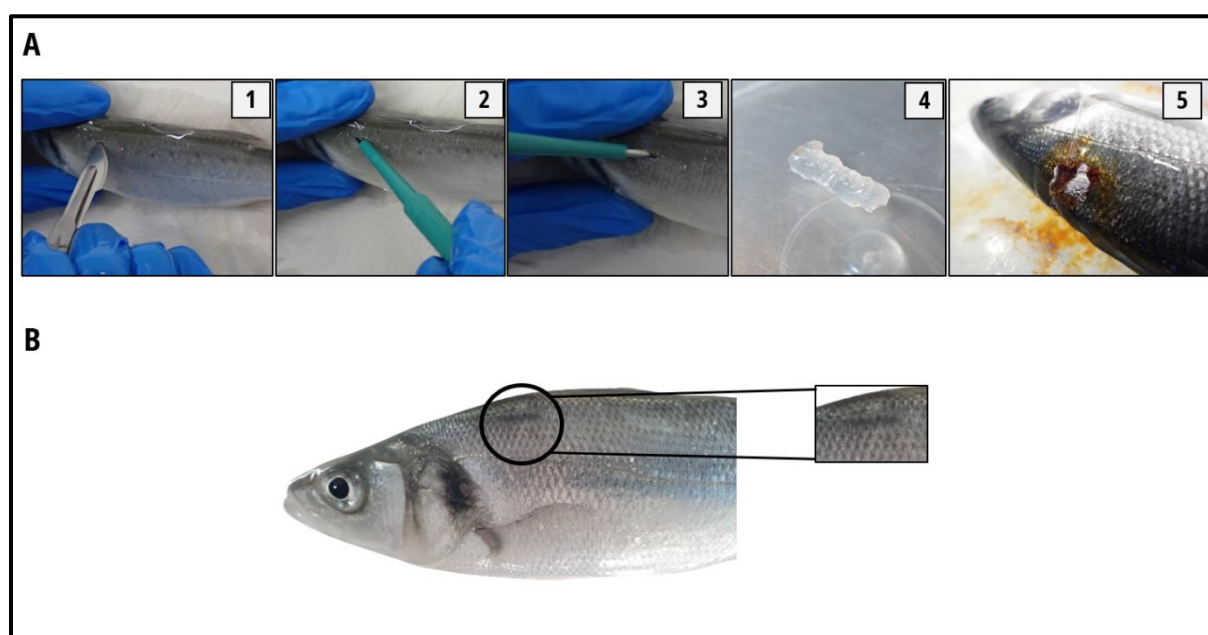
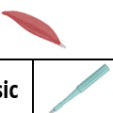





Figure 20: Picture of the procedure for the biopsy (A) and residual scar one month after the biopsy (B). The steps are illustrated in the order given by the numbers on the top right of the pictures. The biopsy is realised on anaesthetised fish by making a small incision on the fish (A1) then inserting the biopsy punch inside the cut (A2) and rotating it to sample a piece of white muscle (A3, 4). Antiseptic solution (Vétédine® Solution, Vetoquinol, Magny-Vernois, France) and surgical glue (ORAHESIVE, ConvaTec®, Deeside, UK) are then applied on the wound (A5).

4. Sample analysis

Table 5: Summary of samples analysed during this thesis

	Type of analysis						Total
		Classic				OIL	
Chapter 1 In-situ study	Fatty acids	50		50	76		176
	Molecular				75		75
Chapter 2 Swimming & Metabolism	Fatty acids	31		31	31	12	105
	Molecular				31		31
Chapter 3 Behaviour	Fatty acids					6	6
Chapter 4 Biopsy	Fatty acids	48	6			6	60

4.1. Fatty acid analysis

4.1.1. Lipid extraction

Extraction of lipids from liver, muscle and brain

Frozen tissues were grounded in liquid nitrogen into a homogeneous powder. For the liver, half of the grounded tissue was used for fatty acid analysis, and the other half was kept and stored at -80°C for molecular analyses. As the liver and the brain contain much more lipids compared to the muscle, 50 mg of powdered brain and liver, and 200 mg of powdered muscle were used for fatty acid analyses, respectively. Lipids were extracted from the powdered tissues by diving them into a 6 mL mixture of chloroform/methanol (Folch, 1957; 2:1, v/v). To optimise the lipid extraction, all of the extracts were sonicated for 10 min, agitated for 20 min, and stored at -20°C under nitrogen atmosphere prior to further analysis (Couturier et al. 2020).

Extraction of lipids from biopsy samples

The lipid extraction of biopsy samples is detailed in the dedicated paper (see chapter 4).

Extraction of lipids from pellets

To extract lipids from pellets, 10 mg of pellets were grounded into a homogenous powder and divided into 6 mL of a mixture of CHCl₃/MeOH (2:1, v/v).

Extraction of lipids from oils

To analyse crude oil samples used to manufacture diet pellets, 300 µL of pure oil was mixed in 6 mL of CHCl₃/MeOH (2:1, v/v) to create a stock solution at 5 mg mL⁻¹. A volume of 100 mL of this stock solution was evaporated to dryness under a nitrogenous flux and re-suspended with 5 mL of CHCl₃/MeOH (2:1, v/v) to create a diluted solution at 0.1 mg mL⁻¹.

4.1.2. Separation of Neutral and Polar lipids

Only the lipids extracted from tissue samples were separated into NL and PL classes. The fatty acids from the oils and pellets were analysed in total lipids (TL).

For all the tissue samples, lipids were separated into a neutral (NL) and polar (PL) fraction following the method described by Le Grand et al. (2014). An aliquot (1/6) of the total lipid extract was evaporated to dryness, re-suspended three times with 500 µL of a mixture of chloroform/methanol (98:2, v/v) and deposited at the top of a silica gel (40 mm x 4 mm, silica gel 60A 63-200 µm rehydrated with 6% H₂O (70-230 mesh)). NL were eluted with 10 mL of a mixture of CHCl₃/MeOH (98:2, v/v) and PL were then eluted with 20 mL of MeOH. After the elution, each fraction that was then evaporated to dryness with a Genevac centrifugal evaporator.

4.1.3. Transesterification

Transesterification consists of the separation of FAs from glycerol through hydrolysis and methylation to obtain fatty acid methyl esters (FAME) that are volatile, to be further analyzed using gas chromatography. FAME were obtained from NL, PL (for tissue samples), and TL (for diets and oils). After evaporation to dryness of the tissue LN and LP fractions, or, of an aliquot of the oil or pellets TL extract, 2.3 µg of an internal standard (tricosanoic acid, C_{23:0}) was

added to 1600 μL of $\text{H}_2\text{SO}_4/\text{MeOH}$ (3.4%) and the samples were incubated for 10 mn at 100°C . 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 with hexane-saturated distilled water.

4.1.4. Fatty acid profiles analysis

The NL and PL fatty acids profiles were analysed by gas chromatography (GC) coupled with a flame ionization detector (GC-FID), as described by Mathieu-Resuge et al. (2019). The GC uses a mobile phase that is a stream of hydrogen gas and a stationary phase that is a liquid held within a capillary column. The principle of the FID is based on the ionization of the molecules in the mixture. As it exits the column, the gas passes through a flame. The molecule eluted by the gas is oxidized by the flame and produces ions. An electrode that generates an electrical signal proportional to the amount of ions formed then detects these ions. A computer processes this signal.

Analyses of FAME were performed on a Varian CP8400 GC-FID using simultaneously two separations on a polar column (DBWAX: 30 mm \times 0.25 mm ID \times 0.2 μm , Agilent) and an apolar column (DB5: 30 m \times 0.25 mm ID \times 0.2 μm , Agilent) using hydrogen as carrier gas with a flow rate set at 2 $\text{mL}\cdot\text{min}^{-1}$, in splitless mode. The oven temperature was programmed as follows: first, initial heating from 60°C to 150°C at $50^\circ\text{C}\cdot\text{min}^{-1}$, then to 170°C at $3.5^\circ\text{C}\cdot\text{min}^{-1}$, to 185°C at $1.5^\circ\text{C}\cdot\text{min}^{-1}$, to 225 at $2.4^\circ\text{C}\cdot\text{min}^{-1}$ and finally to 250°C at $5.5^\circ\text{C}\cdot\text{min}^{-1}$ and maintained for 25 min. Injector temperature was set at 250°C , detector temperature at 280°C .

Chromatogram processing and peak integration were carried out using the Galaxie software v1.9.3.2 (Varian) (Figure 21). The FAMES were identified by comparing their retention times with those of commercial standards (Supelco 37 Component FAME Mix, PUFA No.1 and No.3, and Bacterial Acid Methyl Ester Mix, Sigma) and standard mixtures prepared in the laboratory. The masses of each fatty acid were then calculated relative to the internal standard using the following formula:

$$m = \frac{A_{FA} \times 2.3}{A_{C23:0}}$$

Where m is the mass of the considered fatty acid (μg), and A_{FA} and $A_{C23:0}$ are the respective areas of the peak corresponding to the considered fatty acid and C23:0 (in arbitrary units).

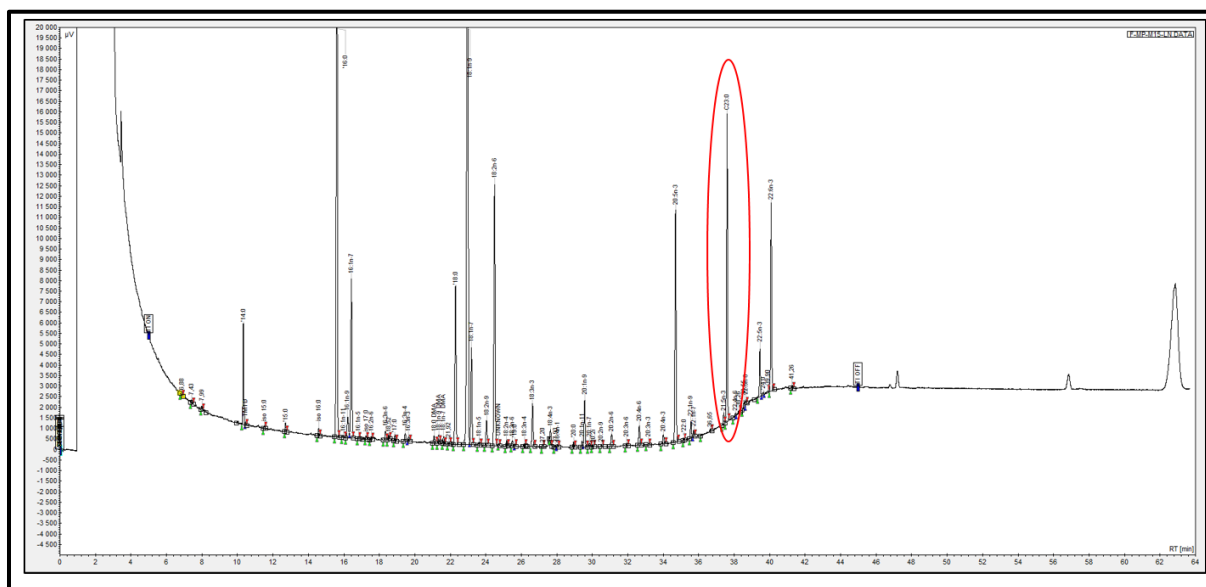


Figure 21: Chromatogram of muscle PL FA obtained with GC-FID. The red ellipse shows the internal standard (C23:0) used to obtain the masses of each FA.

4.2. Gene expression analysis

4.2.1. Selection of genes

The genes of interest were partly selected during the Master 2 Internship of Sarah Hue (2020). All the genes that were analysed during this thesis are presented in **Table 6**. For all the genes (except *fas*), the sequences used were those described by Rimoldi et al. (2016) that validated them on the European sea bass. For *fas*, we found the mRNA and cDNA sequences for European sea bass in the GENBANK database (<https://www.ncbi.nlm.nih.gov/genbank/>). For each gene of interest, a sequence ranging from 150 to 200 base pairs (bp) was established to design primers. These primers, of about 20 bp, should neither be complementary to one another nor possess secondary structures. Furthermore, their sequences should predominantly contain guanine (G) and cytosine (C), ideally comprising 60% of the total sequence.

4.2.2. RNA Extraction

Total RNA were extracted from approximately 30 mg of liver frozen powder using Extract-all reagent (Eurobio; Courtaboeuf, Essonne, France) coupled with purification steps on a Nucleospin RNA column that includes one step of DNase treatment and according to the manufacturer instructions (Macherey–Nagel, Düren, Germany). Concentrations and purity of extracted RNA were measured using a ND-1000 NanoDrop spectrophotometer (ThermoScientific Inc., Waltham, MA, USA). The ratio between the two measured optical densities (260/280) should fall between 1.6 and 2. This ratio is used to assess the purity level of the extracted total RNA pool (at 260 nm) and to identify potential protein contamination (at 280 nm). An Agilent Bioanalyzer 2100 or a 4150 TapeStation system (Agilent Technologies Inc, Santa Clara, CA, USA) was used to evaluate the RNA integrity (RIN). The RIN is a numerical scale, ranging from 1 (completely degraded RNA) to 10 (intact RNA), that provides an assessment of the quality of RNA, which is crucial for the success of the subsequent reverse transcription. The RIN is determined by analyzing the ratio of ribosomal RNA subunits and the entire electrophoretic trace of the RNA sample. Only samples showing a $RIN \geq 8$ were considered for analysis. Total RNA samples were then stored at -80°C until use.

4.2.3. Reverse transcription

The reverse transcription (RT) is a technique used to convert messenger RNA (mRNA) into complementary DNA (cDNA). This process is made possible by the enzyme reverse transcriptase. RT is crucial for studying gene expression, as it allows for the analysis of mRNA levels in cells or tissues by converting them into a more stable DNA form, which can then be amplified and analyzed through techniques like polymerase chain reaction (PCR). Here, RT of complementary cDNA for all samples was carried out using 500 ng of mRNA with an iScript™ cDNA Synthesis kit (Bio-Rad Laboratories Inc., Hercules, CA, USA) following strict recommendations from the supplier. cDNA was stored at -20°C until use.

Table 6: GENBANK accession numbers and specific primers of the sequences used for each gene.

Gene	Forward primer (5'->3')	Reverse primer (5'->3')	Accession number
Target Genes			
fads2	CCGCCGTGACTGGGTGGAT	GCACAGGTAGCGAAGGTAGTAAGACATAGA	EU647692
lpl	CAATGTGATCGTGGTGGACTG	CGTCGGGTAGTGCTGGTT	AM411614
plag12b	TTGAGTCAGTTGGCGGCTACTT	CGGTATTGGCACACTCCATCAC	KF857301
scd1b	GCTTGTGGCATACTTCATCCCTGGACTC	GGTGGCGTTGAGCATCACGGTGTA	FN868643
sdhc	ACATGGGCAAGGGCTTCAAA	CGATGATGGACAGACCGATAACG	KF857305
fas	CTAACAACATCTGCCAGCG	GACGGTGGCAGAATTGGATC	MF566098
House Keeping genes			
ef1 α	CTGGAGGGCAGTGAAAAGAT	CATCAAGAGCCTCCAGCAGT	AJ866727
act	GTGCGTGACATCAAGGAGAA	AGACGCACAATCTTGAGAGCAG	AY148350
l13a	GCTTCGAGGAAATCACCAAG	CAACCTTCCATCCCTTGAAC	DT044539

4.2.4. Quantitative PCR (qPCR)

Quantitative Polymerase Chain Reaction (qPCR), also known as real-time PCR, is a laboratory technique used to amplify and simultaneously quantify targeted DNA molecules. It allows for the detection of specific DNA sequences using primers (one forward, and one reverse), that each bind on their complementary strand of DNA, preliminary denatured, then it monitors the amplification process in real-time through the use of fluorescent markers. Housekeeping genes, which are consistently expressed in various cell types and under different physiological conditions, were used as internal controls for normalization of the expression of the target

genes. These housekeeping genes are crucial for maintaining fundamental cellular functions and exhibit stable expression levels, making them reliable references. Their expression levels serve as a baseline, facilitating accurate comparisons of target gene expression levels across different samples. The selected housekeeping genes for this study were *ef1α*, *actin* and *l13α* (Table 6). Gene expression was measured using the CFX96 Touch Real-Time PCR Detection instrument from Bio-Rad Laboratories Inc. We tested each sample in triplicates in a 15 μL reaction volume, which included 5 μL of cDNA (diluted 1/30), and a 10 μL reaction mixture. This mixture consisted of 0.5 μL of each primer (10 mmol L⁻¹), 1.5 μL of RNase/DNase-free water, and 7.5 μL of iQ™ SYBR® Green Supermix from Bio-Rad Laboratories Inc, as a fluorescent marker. The cDNA dilution was chosen after performing a series of diluted cDNA samples, ranging from 1/5 to 1/200 (Figure 22).

The qPCR protocol consisted in:

- A 2 min activation at 95°C to activate the DNA polymerase

40 cycles of:

- 5 sec at 95°C to denature the double stranded DNA into two simple stranded DNA
- 20 sec at 60°C to hybridize the primers, and elongate DNA

CT/CQ values, or Cycle Threshold/Cycle quantification values, represent the number of cycles at which the real-time measured fluorescence emission surpasses the background noise threshold. Essentially, this is the point where the amount of amplified DNA (amplicon) becomes significant and distinguishable from the background noise. The CT value for every sample was automatically calculated using the CFX Manager software's Gene Expression Module from Bio-Rad Laboratories Inc. (Figure 23). The analysis of the melting curves (Figure 24) confirmed that only a single amplicon was amplified.

The qPCR efficiencies (E) refer to the efficiency with which the DNA template is amplified during each cycle of the qPCR. It was calculated using the formula:

$$E = (10^{(-1/slope)} - 1) \times 100$$

Where the slope is derived from the log-linear portion of the amplification curve. All of the efficiencies were above 90%. The relative expression level of the target gene is calculated using the formula. $2^{-\Delta\Delta CT}$. This value represents the fold change in gene expression of the

target gene in the experimental sample relative to the control sample, normalized to the housekeeping gene (Livak and Schmittgen 2001).

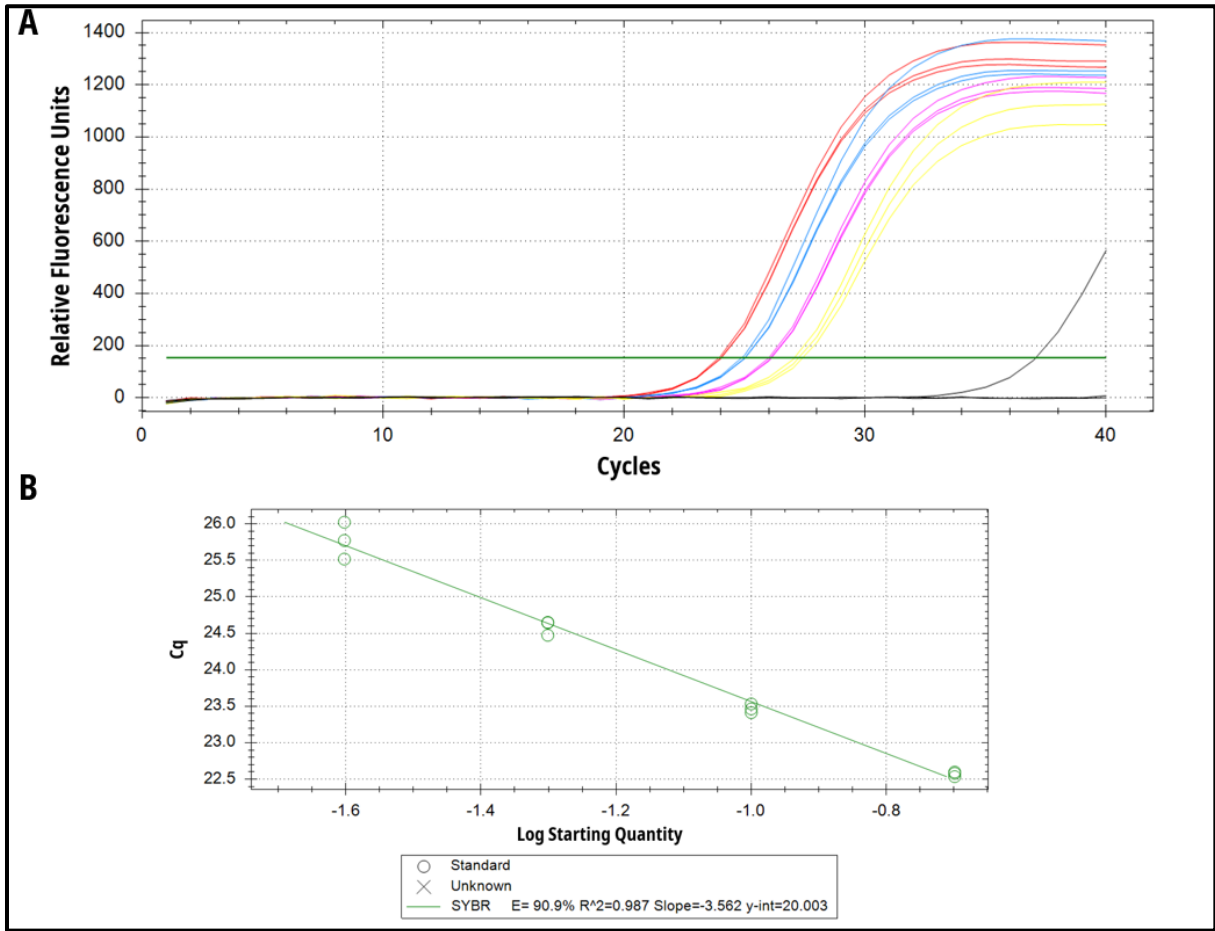


Figure 22: Amplification (A) and standard curve (B) obtained after 4 different dilutions (red, blue, pink, yellow curves corresponding to the dilution 1:5, 1:10, 1:20, 1:40, respectively), of cDNA for the *elov16* gene, performed in triplicates. The last grey curves (A) represent the blank samples.

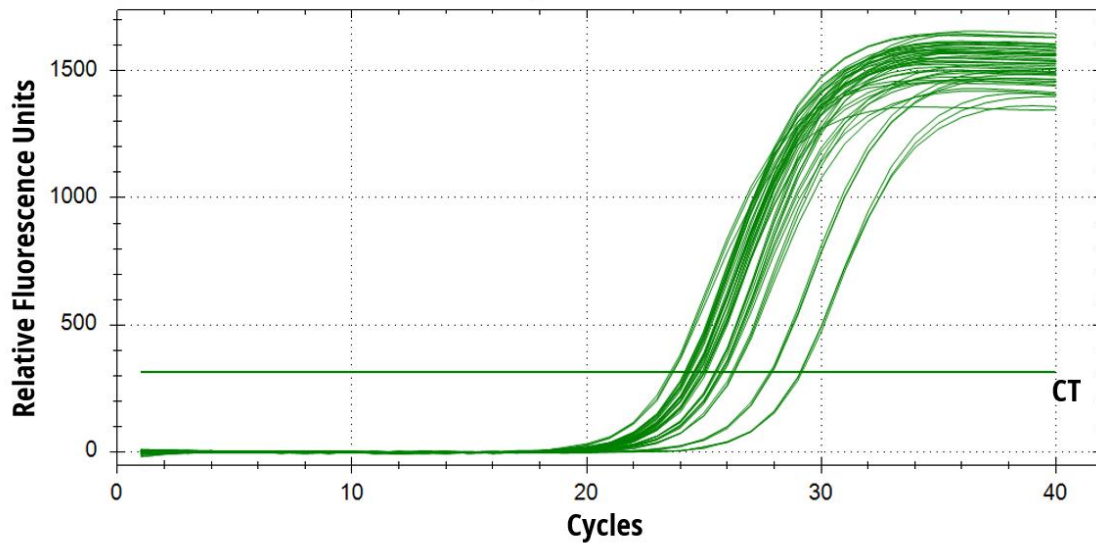


Figure 23: Amplification curve obtained after 40 qPCR cycles for *elovl6* on all the samples performed in triplicates. The CT line was automatically determined.

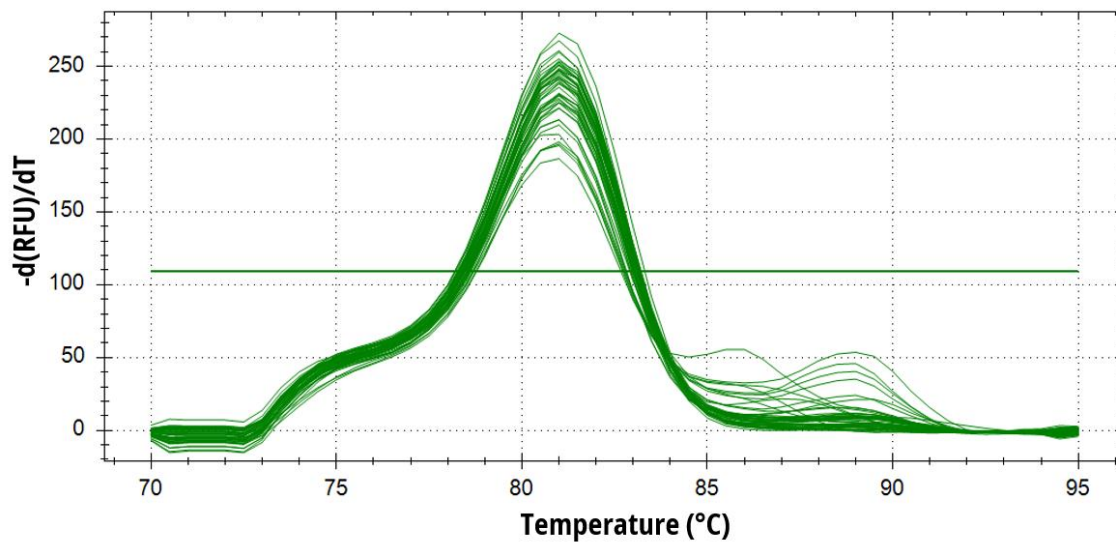


Figure 24: Melting curve after amplification (40 cycles) of *elovl6* in all the tissue samples performed in triplicates. The fluorescence variation ($-d(RFU)/dT$) is expressed as a function of temperature (°C). The unique peak indicates a unique amplicon.

5. *Statistical analysis*

All statistical analyses were realised using RStudio (V4.2.1). For each chapter, the detailed statistical analysis is indicated in the specific Material and Methods section.

***Chapter 1 - Spatial and
ontogenetic modulation of fatty
acid composition in juvenile
European sea bass
(*Dicentrarchus labrax*) from two
French estuaries***

Research paper n°1 entitled “Spatial and ontogenetic modulation of fatty acid composition in juvenile European sea bass (*Dicentrarchus labrax*) from two French estuaries”

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In review for Marine Environmental Research

1. Abstract

This study compared the fatty acid (FA) composition in the liver, muscle, and brain tissues of wild European sea bass juvenile from two French estuaries (Loire and Seine), focusing on the variability across ontogenetic stages (first, second and third year, i.e. G1, G2, G3, respectively). The main saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) were identified as palmitic acid (16:0), oleic acid (18:1n-9), and docosahexaenoic acid (DHA; 22:6n-3), respectively. We highlighted tissue-specific patterns, with the brain exhibiting a distinct FA composition from the two other tissues. Ontogenetic stage and estuary influenced the general FA profile, and particularly essential FAs (EFA) like DHA, eicosapentaenoic acid (EPA), and arachidonic acid (ARA) in all tissues. The data also revealed potential dietary EFA deficiency in the natural environment, especially for Seine G1 juveniles. The differential distribution of FA within tissues might reflect shifts in diet, metabolic demands, or adaptations to environmental conditions. The study provides valuable insights about FA dynamics in euryhaline fish during crucial juvenile life stages, enhancing our understanding of their metabolic and trophic interactions, and underscoring the need to further investigate potential effects of FA depletion in a changing trophic environment.

Key words: Fatty acids, European sea bass, Estuary, Ontogenetic stages, DHA, EPA, ARA

2. Introduction

Long-chain polyunsaturated fatty acids (≥ 20 carbon atoms, LC PUFA), particularly eicosapentaenoic acid (EPA; 20:5-n-3), docosahexaenoic acid (DHA; 22:6n-3), and arachidonic acid (ARA; 20:4n-6), are necessary for numerous biological functions in organisms ranging from the smallest marine phytoplankton to humans (Cottin et al. 2011). They are the major components of cell membrane phospholipids (polar lipids, PL), influencing membrane fluidity, permeability, and functionality (Stillwell and Wassall 2003). They are also found in lesser proportions in triglycerides (neutral lipids, NL), which are a source and reserve of energy. LC PUFA are also precursors of several bioactive molecules. For instance, EPA and ARA serve as substrates for eicosanoid synthesis, a group of potent signaling molecules that mediate numerous physiological processes, including inflammation, immune response and reproduction (Rowley et al. 1995; Calder 2017). 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 (Cunnane et al. 1993) while the muscle, essential for locomotion, usually exhibits high levels of EPA (Tocher 2003). Studying NL and PL fatty acid composition can give insights about how storage and structural lipids are regulated in tissues, and can be linked to physiological performances within an individual (Twining et al. 2020).

LC PUFA, and especially LC n-3 PUFA are naturally mainly synthesized by marine microalgae, the primary producers in marine ecosystems, and are then introduced at the basis of the food chain (Dalsgaard et al. 2003). Fish, as consumers of higher trophic level, rely on the dietary supply of these nutrients due to their limited LC n-3 PUFA biosynthetic capacity. Typically, the conversion of α -linolenic acid (ALA) to EPA and DHA in marine fish occurs at less than 5% (Tocher 2010b). The enzymes involved in this process are the fatty acyl desaturases (*fads*) and elongases of very long-chain fatty acids (*elovl*), which catalyze the conversion of shorter-chain polyunsaturated fatty acids into longer-chain forms (Monroig et al. 2010) (Figure S1). In fish, the liver is the main organ in which lipid biosynthesis occurs, but both liver and brain have shown few LC-PUFA biosynthesis abilities (Monroig et al. 2018b; Galindo et al. 2021). The biosynthesis of LC PUFA in fish is modulated by several environmental factors, including

temperature (Tocher et al. 2004), salinity (Zheng et al. 2005a) 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, emphasizing the plasticity of their metabolic pathways (Vagner et al. 2007b; Glencross 2009). However, this upregulation at molecular level has not been shown to be sufficient to compensate for dietary deficiency in the tissue FA composition (Vagner et al. 2007a, 2009)

Global changes, encompassing factors such as warming, acidification, and hypoxia, 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 (Poloczanska et al. 2013; Galloway and Winder 2015b; Hixson and Arts 2016b). This would lead to a lower LC PUFA availability for their consumers, such as fish, and therefore limiting their ability to adjust the LC-PUFA composition of their membrane (Brett et al. 2009). A reduced LC PUFA content in fish cell membranes has been found to have profound cascading effects on fish physiology, including reduced growth (Vagner et al. 2014) altered energy metabolism, reduced immune function, and impaired reproductive success (Schmitz and Ecker 2008; Bell and Koppe 2010; Vagner et al. 2014, 2015, 2019).

The consequences of these physiological changes may translate to population level, affecting ecosystem structure and functioning (Pauly et al. 2002).

The European sea bass (*Dicentrarchus labrax*) is a key species in the coastal and estuarine ecosystems of the Atlantic Ocean and Mediterranean Sea. The sea bass plays a significant ecological role and supports important commercial and recreational fisheries (Pérez-Ruzafa and Marcos 2014). 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 (Dorel 1986; Pawson et al. 2000). European sea bass is an euryhaline and eurythermal species, capable of thriving in a wide range of salinity and temperature conditions (3-32°C, 0.5-60 salinity, Barnabé, 1990; Chervinski, 1974). 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 (Pickett and

Pawson 1994). As fish mature, their dietary preferences shift, and they might target larger prey potentially having different FA compositions, which is influencing the FA profile of the fish. Juveniles have important nutritional demands, and alterations in the dietary availability in LC PUFA can have profound effects on their survival, growth and fitness (Bell and Tocher 2009). The LC-PUFA metabolism of this species has been extensively studied in the context of aquaculture production and most notably for the replacement of fish oil in aquafeed by vegetal oils (Montero et al. 2005; Mourente and Bell 2006; Vagner et al. 2007a, b; Geay et al. 2010b; Torrecillas et al. 2017a).

Despite this intensive research, little is known about the LC-PUFA metabolism of wild individuals, most of the research being focused on the comparison between wild and farmed sea bass (Orban et al. 2003; Fuentes et al. 2010; Bhourri et al. 2010; Tarricone et al. 2022).

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

Moreover, 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).

Addressing the knowledge gaps concerning the capacity of fish to adjust to changing environmental conditions and the potential consequences for ecosystem structure and function is of paramount importance. Considering the crucial role of FAs in organisms,

studying their distribution across various organs in fish at different ontogenetic stages and from different estuaries can provide valuable insights about their dietary preferences and physiological adjustment capacities in complex environmental conditions. Such knowledge would contribute to the understanding of FAs dynamics in fish and enlighten us about possible challenges that fish will have to face in the context of a changing environment.

In this context, the present study aims to investigate the influence of life history traits (ontogenetic stage and estuary) on the (i) LC PUFA profiles of the liver, muscle and brain of juvenile wild sea bass in the Seine and Loire estuaries; (ii) modulation of LC n-3 PUFA biosynthesis pathways at a molecular level and how this is related to the fish FA composition in natural environment.

We expected that (1) different organs will have distinct FA profiles linked to their physiological roles, (2) that the ontogenetic stage and estuary of origin will influence these profiles in tissues, having metabolic needs and foraging preys depending on the age and that (3) there is relationship between LC PUFA profiles and expression of genes involved in lipid metabolism.

3. Material and Methods

3.1. Studied sites and sample collection

Juvenile European sea bass were sampled in the Seine and Loire estuaries in July and August 2019, respectively, during an annual NOURDEM survey funded by Ifremer (French Institute for Sea research and Exploitation). Samplings were performed over a period of 2 to 5 days (for zones of capture, see [Figure 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. The collected sea bass were then euthanized using MS-222 (400 mg.L⁻¹). We measured fish total length (nearest 0.1 cm), weight (nearest gram) and sampled a few scales to confirm the age of the different fish. Brain and liver were entirely removed. About 200 mg of muscle were taken on the left side of the fish, dorsally from the lateral line and just

behind the head. Gallbladder was removed from the liver. All samples were immediately flash frozen in liquid nitrogen until reaching the lab, where they were stored at -80°C pending further analysis. A total of 76 individuals were collected: 12 for Seine G1 (SG1), 18 for Loire G1 (LG1), 10 for Seine G2 (SG2), 12 for Loire G2 (LG2), 12 for Seine G3 (SG3) and 12 for Loire G3 (LG3).

3.2. Life history traits measurements

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

$$K_n = W/aL^b$$

Where 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.

3.3. FA profiles analysis

3.3.1. 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 7](#)) 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 7: 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

3.3.2. Lipid separation

For all the samples, lipids were separated into a neutral (NL) and polar (PL) fraction following the method described by Le Grand et al. (2014). An aliquot (from 750 to 3000 μ L, depending on the sample biomass) of the total lipid extract was evaporated to dryness, re-suspended three times using 500 μ L of a mixture of chloroform/methanol (98:2, v/v) and deposited at the top of a silica gel (40 mm x 4 mm, silica gel 60A 63-200 μ m rehydrated using 6% H₂O (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 μ g 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 μ L of H₂SO₄/MeOH (3.4%) were added and the samples were incubated for 10 mn at 100°C to form FA methyl esters (FAME). FAMES were extracted by adding 800 μ L of hexane and 1500 μ L 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.

3.3.3. FAME analysis

FAMES were analyzed in a Varian CP8400 gas chromatograph (GC) coupled with flame ionization detector (FID) as described in Mathieu-Resuge et al., (2019). FAMES were injected in splitless-mode in parallel on two different columns (DBWAX 30m x 0.25 mm ID x 0.2 µm and DB5 30 m x 0.25 mm ID x 0.2 µm, Agilent). Identification of FAME was realized by comparison of their retention times based on those of commercial standards (Supelco, 37 Component FAME mix, PUFA N°1 and N°3, and Bacterial Acid Methyl Ester Mix, Sigma). Internal standard allowed calculating 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.

3.3.4. Gene expression analysis

Total RNA were extracted from the liver powder (n=75; see [Table 7](#) 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 Bionalyzer 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 S1](#)): Fatty acid desaturase 2 (*fads2*), Lipoprotein lipase (*lpl*), Group XIIB secretory phospholipase A2 (*plag12b*), Stearoyl-CoA desaturase 1b (*scd1b*) and Succinate dehydrogenase cytochrome b560 subunit (*sdhc*). These genes were chosen because they are involved in the lipid or LC-PUFA metabolism (Rimoldi et al. 2016). The primers used, as well as the GENBANK sequence numbers are presented for each gene in [Table S1](#). 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 $\Delta\Delta C_t$ method (Livak and Schmittgen 2001).

3.4. Statistical analysis

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

4. Results

For both estuaries, an increase in mean fish weight and length was observed from G1 to G3 (Table 8). However, the Loire fish consistently had a greater weight and length than Seine fish at each stage. These differences were statistically significant for both stage ($p < 0.001$) and estuary ($p < 0.001$). The CF, however, remained constant across developmental stages for both estuaries and no significant differences were observed in relation to either the stage or estuary.

Chapter 1 - Spatial and ontogenetic modulation of fatty acid composition in juvenile European sea bass (*Dicentrarchus labrax*) from two French estuaries

Table 8: Weight (g), Total Length (TL, cm) and LeCren condition Factor (Kn) of the juvenile European sea bass from the different ontogenetic (G1: one year old; G2: two years old; G3: three years old) and location (Seine and Loire estuaries) groups. Values are expressed as mean \pm 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 \pm 2.9 ^b	162.7 \pm 6.2 ^d	307.4 \pm 17.9 ^f	38.6 \pm 2.8 ^a	137.5 \pm 6.0 ^c	270.1 \pm 15.2 ^e	***	***	-
TL	16.5 \pm 0.3 ^b	25.3 \pm 0.3 ^d	31.1 \pm 0.6 ^f	15.3 \pm 0.4 ^a	23.9 \pm 0.4 ^c	29.8 \pm 0.5 ^e	***	**	-
K _n	0.96 \pm 0.04	1.01 \pm 0.03	1.01 \pm 0.01	1.08 \pm 0.02	1.01 \pm 0.02	1.01 \pm 0.02	-	-	-

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) (**Figure 25A**). The brain displayed the highest average monounsaturated fatty acid (MUFA) concentration (30 \pm 0.4%) in PL among tissues, which was more than twice that of the liver and muscle (14 \pm 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 \pm 0.2%) (**Figure 25A**). 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 (**Figure 25A**).

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%), MUFA (32%), and PUFA (33%) with 16:0, 18:1n-9, EPA and DHA as major FAs in both tissues. Liver had the highest concentrations of MUFA (40%; **Figure S2B**) with 18:1n-9 being the major FA (**Figure 25B**).

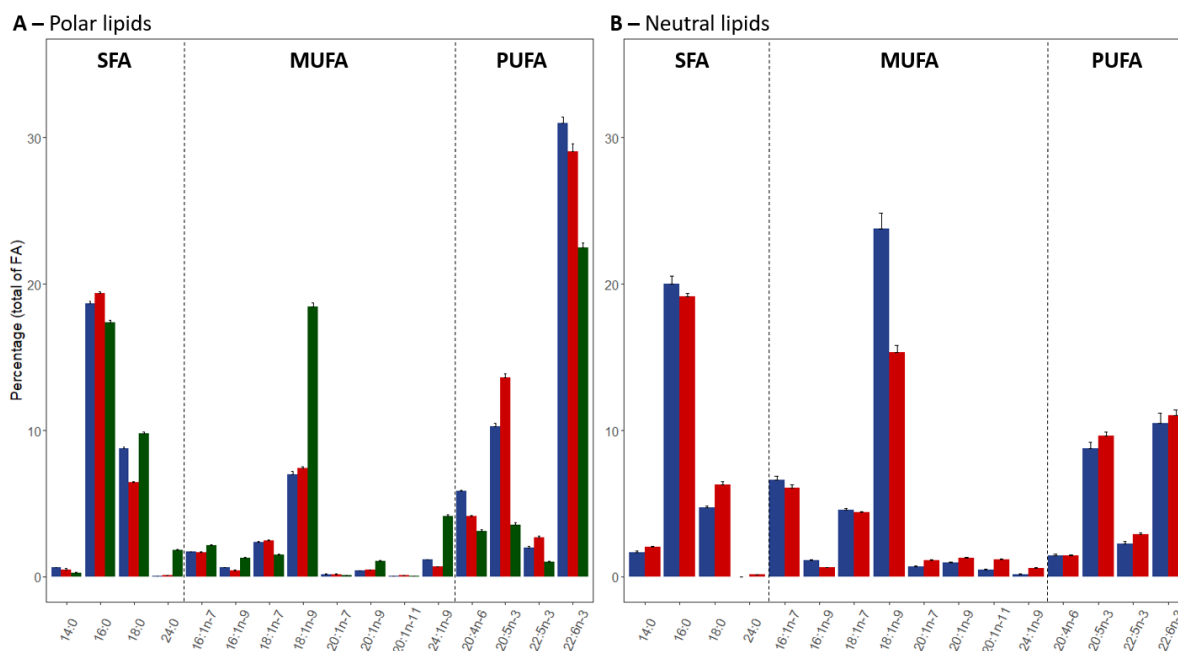


Figure 25: Proportions of fatty acids (mass percentage of total FA) in polar lipids (A) and neutral lipids (B) in the liver (blue, n=75), muscle (red, n=50) and brain (green, n=49) of juvenile European sea bass from all confounded Seine and Loire locations and ontogenetic stages. Only FA that are >1% for at least one tissue are presented. Data are presented as mean \pm SEM. Neutral lipids have not been measured in the brain (cf material and method section for details). SFA = Saturated Fatty Acid, MUFA = Monounsaturated Fatty Acid, PUFA = Polyunsaturated Fatty Acid.

The inertia of FA composition in fish PL, according to their ontogenetic stage and sampling location is presented in the PCA in **Figure 26**.

In the liver (**Figure 26A**), significant differences in PL FA composition were found among the groups (PERMANOVA, **Table S3**, **Table S4**). In the Seine estuary, the G1 from Seine were different from the two other ontogenetic groups and appeared to be distinguished, among others by their EPA (20:5n-3) proportions, while G2 and G3 seemed to be distinguished by

their DHA (22:6n-3) proportions (**Figure 26A**). However, that trend was not observed in the Loire estuary.

In muscle (**Figure 26B**), significant differences were found among the groups (PERMANOVA, **Table S3, Table S4**). The Seine G1 group was different from all the other groups except from Seine G2. The first dimension distinguished the stages, with G1 being characterized by EPA and G2 and G3 being characterized by DHA. The second dimension distinguished the estuaries, with Seine that tended to be characterized by 16:0 and 18:0, and Loire by 22:5n-6 and ARA (20:4n-6). Brain FA composition in PL (**Figure 26C**) was impacted by an interactive effect between site and ontogenetic stages, with the Seine G2 group being different from Loire G1 and Loire G2 (PERMANOVA, **Table S3, Table S4**). The G1 seemed to be distinguished by EPA and DPA (22:5n-3) and the G3 by ARA and 16:1n-9.

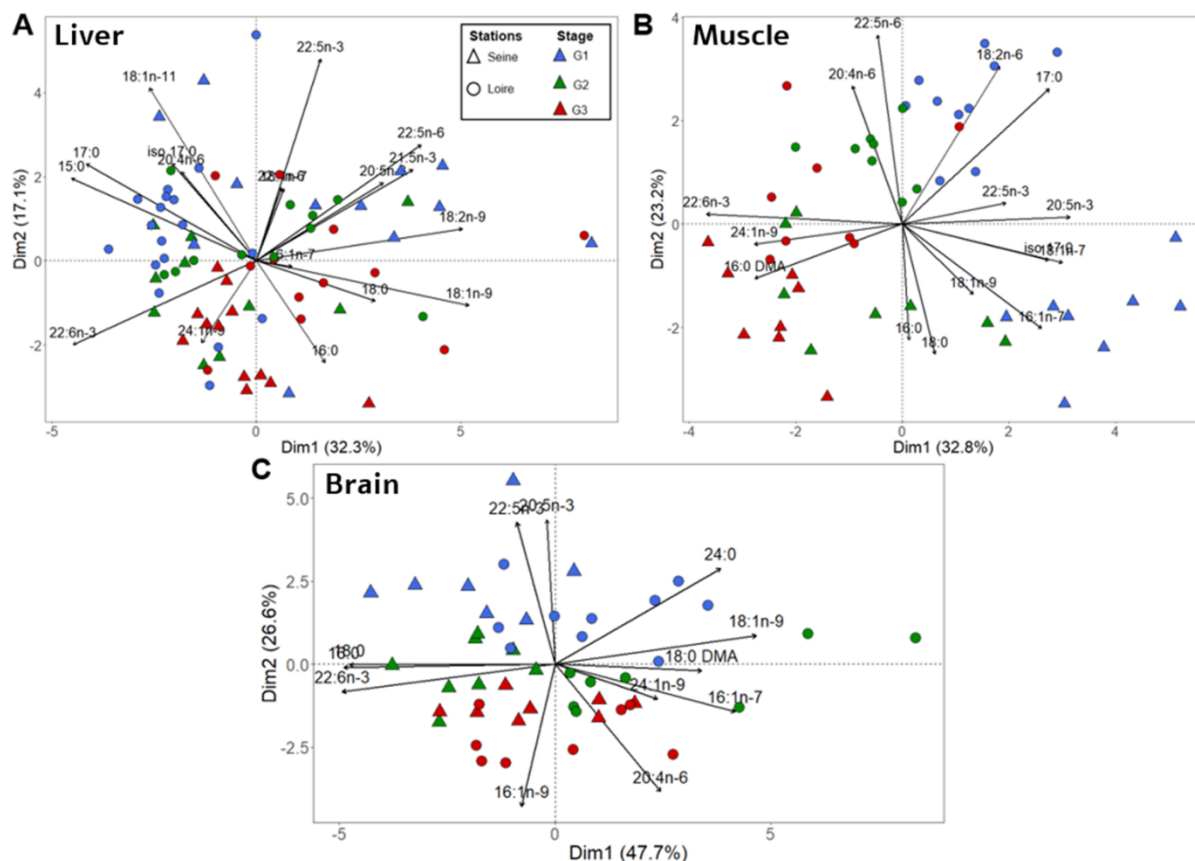


Figure 26: Principal Component Analysis (PCA) of polar lipid fatty acids in liver (A), muscle (B) and brain (C) of 1 (G1), 2 (G2) or 3 (G3) years old juvenile European sea bass from Seine and Loire. Only FAs that account for >80% of the contribution of dissimilarity between groups are shown (SIMPER test) Liver: Loire G1 (n=18), Loire G2 (n=11), Loire G3 (n=12), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12). Muscle : Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=8), Seine G2 (n=8), Seine G3 (n=8). Brain: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=7), Seine G2 (n=8), Seine G3 (n=8).

4.1. FA proportions and ratios between ontogenetic stage and estuaries

4.1.1. DHA proportions

In the liver (**Figure 27A**), DHA proportions followed different dynamics in the two estuaries. It decreased with the ontogenetic stage in Loire, while it tended to increase in Seine. In muscle (**Figure 27B**), DHA significantly increased with the ontogenetic stage in Seine, while it remained similar in all ontogenetic groups in Loire. Seine G1 displayed a lower DHA proportion than all other groups. In the brain (**Figure 27C**), the DHA content did not differ among the ontogenetic stage in Seine. The G2 in Loire had a lower DHA content than the G3 in Loire and was lower than all ontogenetic stages of Seine.

4.1.2. EPA proportions

For the liver (**Figure 27A**) in Loire, the EPA proportions followed an opposite pattern to that of DHA, with Loire G3 having higher proportions compared to Loire G1. It was also significantly higher than in Seine G2 and Seine G3. However, EPA proportions remained stable in groups of Seine. In muscle and brain (**Figure 27B, Figure 27C**), EPA proportions were significantly impacted by ontogenetic stages similarly in both estuaries, with significantly higher EPA proportions in G1 than in G2 and G3.

4.1.3. DHA/EPA ratios

In the liver (**Figure 27A**), the DHA/EPA ratio followed the same trend as the DHA proportions (**Figure 27A**). In muscle (**Figure 27B**), DHA/EPA ratio increased from G1 to G3 in both estuaries. In the brain (**Figure 27C**), the DHA/EPA ratio was not different among the Seine groups, while in Loire it was lower for G1 and G2 compared to G3.

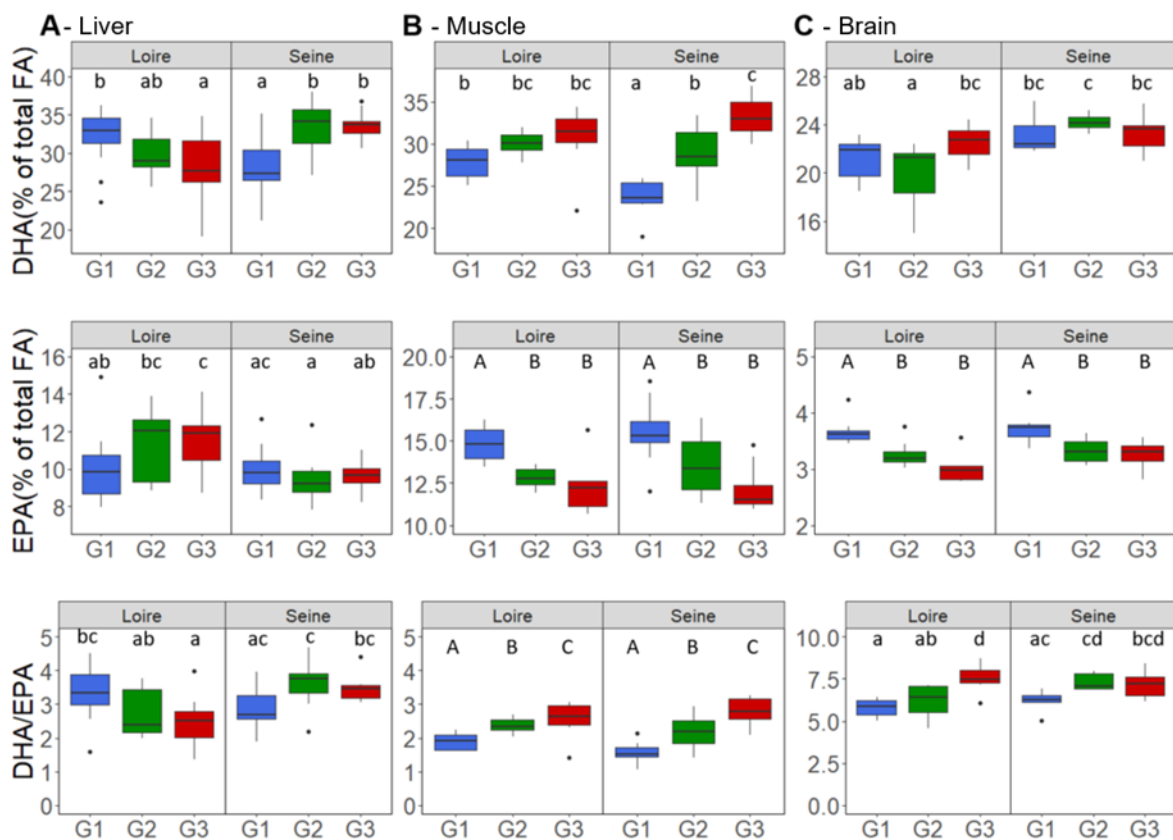


Figure 27: Proportions of DHA and EPA (percentage of total FA) and DHA/EPA ratio in the polar lipids of liver (A), muscle (B) and brain (C) of juvenile European sea bass from Seine and Loire. Different

letters within a plot indicate significant differences. Letters in lowercase represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc) and uppercase letters represent a significant difference between either stage or estuary without significant interaction. Significance was accepted at $p < 0.05$. Liver: Loire G1 (n=18), Loire G2 (n=11), Loire G3 (n=12), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12). Muscle: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=8), Seine G2 (n=8), Seine G3 (n=8). Brain: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=7), Seine G2 (n=8), Seine G3 (n=8).

4.1.4. ARA proportions

In all tissues, ARA proportions were higher in Loire than in Seine. In the liver (**Figure 28A**), ARA proportions were higher in G2 than G1 in both estuaries. In muscle (**Figure 28B**), it did not differ between ontogenetic stages in any estuary, while in the brain (**Figure 28C**), it increased consistently with stages in both estuaries.

4.1.5. EPA/ARA ratios

In the liver (**Figure 28A**), no statistical difference was found for the EPA/ARA ratio. In the muscle and brain (**Figure 28B, Figure 28C**), the EPA/ARA ratio was higher in the Seine than in Loire and G1 had a higher ratio than G2 and G3.

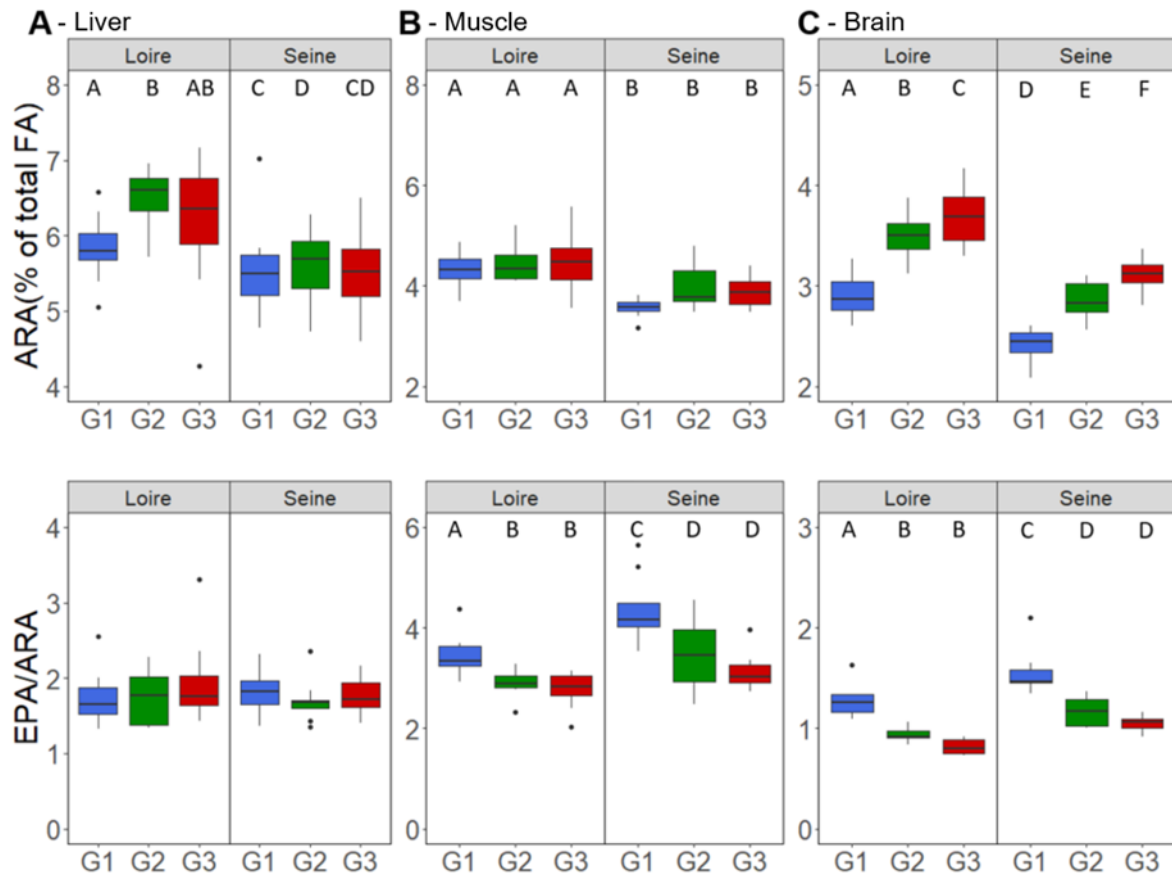


Figure 28: ARA proportions (percentage of total FA) and EPA/ARA ratios for liver (A), muscle (B) and brain (C) of juvenile European sea bass from Seine and Loire. Different letters indicate significant differences. Letters in lowercase represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc) and uppercase letters represent a significant difference between either stage or estuary without significant interaction. Significance was accepted at $p < 0.05$. Liver: Loire G1 (n=18), Loire G2 (n=11), Loire G3 (n=12), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12). Muscle: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=8), Seine G2 (n=8), Seine G3 (n=8). Brain: Loire G1 (n=10), Loire G2 (n=8), Loire G3 (n=8), Seine G1 (n=7), Seine G2 (n=8), Seine G3 (n=8).

4.1.6. Gene expression

The *fads2* and *scd1b* gene expressions followed the same pattern in all groups (**Figure 29A**, **Figure 29C**). Their highest expressions were measured in G1 from Seine, while their lowest expressions were measured in the G1 and G2 from Loire, as well as in the G3 from Seine. Negative correlation between DHA and *fads2* expression was observed for the G1 Seine group as well as significant correlation between FA of the n-3 series (**Figure S3**). The *lpl* gene expression did not differ significantly among ontogenetic stages, but significantly differed between the two sites, and were being globally lower in the Loire groups than in the Seine groups (**Figure 29C**). The *sdhc* and *plag12b* gene expression were not significantly different between groups.

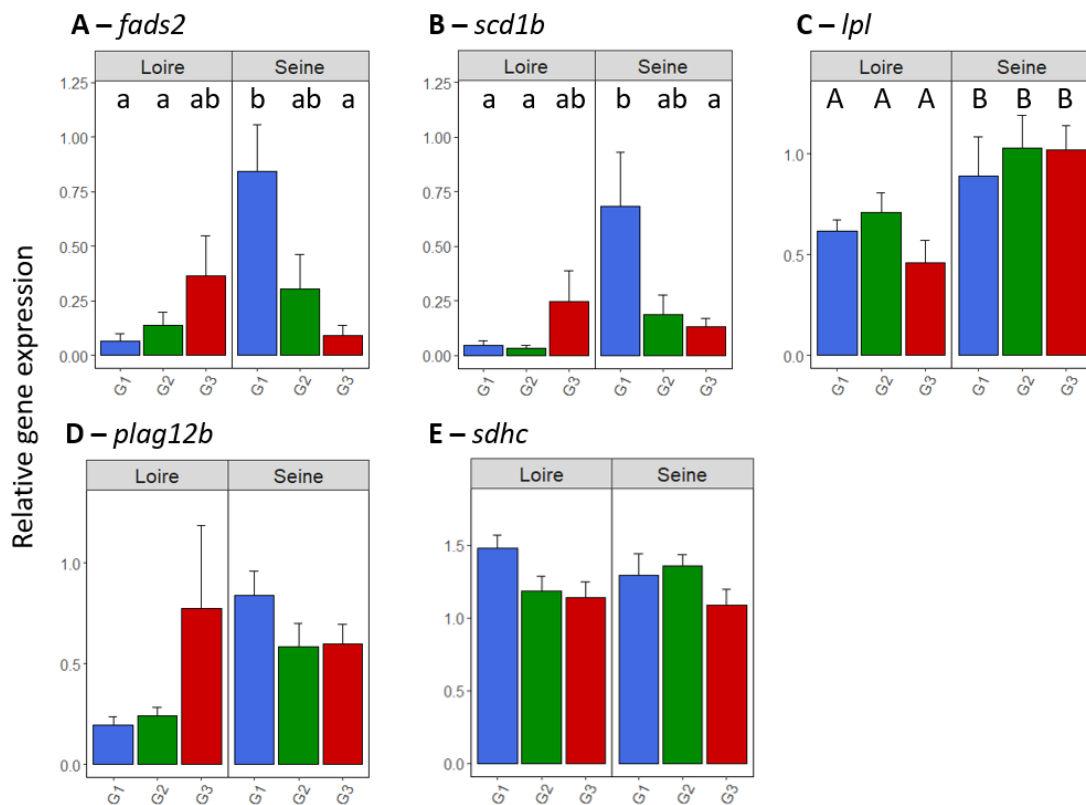


Figure 29: Relative expression of genes coding for enzymes involved in lipid metabolism in the liver of juvenile European sea bass from Loire and Seine, according to their ontogenetic stage (G1, G2, G3, corresponding to first, second and third years old, respectively). The *fads2*, fatty acid desaturase 2 (A), *lpl*, lipoprotein lipase (B), *plag12b*, group XIIIB secretory phospholipase A2 (C), *scd1b*, stearoyl-CoA desaturase 1b (D), *sdhc*, succinate dehydrogenase cytochrome b560 subunit (E). Different letters indicate significant differences among groups. Letters in lowercase represent a significant interaction between the estuary and the stage (2-way ANOVA and Tukey's post hoc), and uppercase letters represent a significant difference between either stage or estuary without significant interaction. Significance was accepted at $p < 0.05$. Loire G1 (n=15), Loire G2 (n=12), Loire G3 (n=11), Seine G1 (n=12), Seine G2 (n=10), Seine G3 (n=12).

5. Discussion

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

proportions were also influenced by estuarine environment and ontogenetic stages. At molecular level, the activation of LC PUFA biosynthetic pathways was the highest in the group that had the lowest DHA proportions in liver and muscle (Seine G1).

5.1. FA profile of juvenile European sea bass

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

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

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

The muscle tissue also showed variation among both ontogenetic stages and estuaries. This might indicate specific dietary preferences or prey availability, reflecting the distinct trophic systems of each estuary (Darnaude et al. 2004). Indeed, some FAs are considered as trophic markers and can be used to identify certain primary producers (Dalsgaard et al. 2003). Seine G1 FA profiles were distinguished by high proportions of 16:1n-7, a diatom FA trophic marker (Cañavate et al. 2019) and Loire G1 FA profile were distinguished itself by high proportions of 18:2n-6, a cryptophyte trophic marker (Viso and Marty 1993). This could indicate different primary production between each estuary. Additionally, the differences in FA composition observed between ontogenetic stages may be indicative of distinct life stages dependent metabolic requirements, as younger fish typically have higher growth rates and metabolic demands (Jobling 1995). It has been suggested that the phospholipid requirements would decrease with development from larvae to juveniles (Tocher et al. 2008) and could have a repercussion on the membranes of juveniles from different ages. Also, the G3 being closer to reproduction, this might have enhanced needs for essential FA (Izquierdo et al. 2001). Interestingly, no discernible pattern of PL FA composition was found in the liver among the groups (G1, G2, and G3) for both estuaries, even if some groups were statistically different from others. As previously discussed, the liver is a primary site for lipid metabolism, and it tends to have a dynamic fatty acid profile reflective of both diet and metabolic regulation (Tocher 2003). This absence of pattern may suggest a quick turn-over of the overall FA profile in the liver (Mohan et al. 2016). Despite the not significant results, brain FA composition showed a trend with EPA discriminating the G1 stage and ARA discriminating the G3 in both estuaries. This could result from trophic difference between ontogenetic groups, as brain FA composition of fish has been proven to be modulated by dietary FA in *D. labrax* (Pagliarani et al. 1986) and gilthead sea bream (*Sparus aurata*, Carvalho et al., 2022) or from different needs for brain development.

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

The tissue-specific DHA proportion measured in wild fish in the present study are in accordance with what is usually observed experimentally in fish from aquaculture when fed a controlled diet (Skalli et al. 2006). The lower DHA proportions in fish liver from Loire G3 could also indicate a reallocation of the DHA from the liver to the growing reproductive organs, as it has previously been shown in zebrafish *Danio rerio* (Zhu et al. 2019). This hypothesis is supported by the greater length of the G3 fish from Loire than those from Seine, likely indicating a closer sexual maturity. It has been reported to happen at a minimum of 32 cm for males in the Atlantic waters (Pawson and Pickett 1996). The increasing DHA content from Seine G1 to Seine G3 in muscle, concomitant with decreased EPA content, could be explained by a shift of diet from zooplankton to diverse epibenthic fauna through ontogenetic stages (Aprahamian and Barr 1985; Pickett and Pawson 1994). Fish as a prey are richer in DHA than invertebrates (*e.g. Mysidacea*) that are richer in EPA (Daly et al. 2010), the older and bigger fish would then incorporate more DHA in their muscle tissue where selective retention happens. Interestingly, the lowest DHA proportion measured in the Seine G1 group was associated with higher relative *fads2* and *scd1b* gene expressions in liver (Figure 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 (Vagner et al. 2007a, 2009; González-Rovira et al. 2009; Geay et al. 2010b). We could thus hypothesize that the lower DHA content associated with the higher *fads2* expression measured in the Seine G1 group may be related to (i) a lower LC-PUFA in their diet, combined to (ii) higher DHA demand due to a higher cellular turnover in this growing ontogenetic stage. Additionally, the mean DHA proportions measured in the liver and muscle of Seine G1 were slightly lower than those reported by Skalli et al., (2006) (28% vs 30%, 23% vs 24%, respectively) in the same species, from aquaculture, and experimentally fed with a low PUFA diet (0.4% EPA+DHA on dry matter basis). The needs have been experimentally established at 0.7% EPA+DHA on dry matter basis for sea bass juveniles (Skalli and Robin 2004). Taken together, these results would support the hypothesis of a dietary limitation in DHA for Seine G1, which would not match to their requirement at this age. Below the

threshold of 0.7% EPA+DHA, growth of the juvenile was negatively affected (Skalli and Robin 2004). However, it is important to consider that this study was conducted in experimental conditions with a goal of optimizing the aquafeed costs for European sea bass farming. Environmental conditions, such as salinity or temperature, can also influence *fads2* expression in teleost fish (for review, see Vagner and Santigosa, 2011), making it difficult to disentangle the reasons underlying the higher *fads2* expression observed in the G1 from Seine. Remarkably, the brain DHA proportion in Seine G1 was maintained as high as in all the other groups, showing likely the preservation of cognitive functions. Despite that, the effects of a possible DHA dietary limitation for this group in the environmental context should not be overlooked as it could impact growth, performances and ability to cope with changing conditions (Bou et al. 2017). The DHA content in the brain was lower in Loire G2 compared to Loire G3 and all ontogenetic stages in Seine. However, this difference was not seen in the muscle or liver. Given that the brain is a stable tissue, as noted by (Carvalho et al. 2022) and (Hong et al. 2014), it's difficult to attribute this difference to a lack of DHA in the diet. Instead, (Skalli et al. 2006) found that *D. labrax* raised at 29°C had lower DHA content than those at 22°C, suggesting temperature affects the FA composition more than diet does.

As observed for DHA, EPA and ARA proportions also displayed a tissue-specific response and differed between estuaries and between ontogenetic stages. However as the pattern observed in the liver was inversely related to that of DHA, this suggests some modulation by trophic interactions or intense metabolic hepatic activity. Similarly, the distribution of EPA in the muscle was opposite to that of DHA, with the youngest individuals displaying the highest levels of EPA. EPA was shown to be an expendable PUFA for the brain in fish unlike DHA who make up for the majority of brain membrane FA and have a proven role in neural functions (Trushenski et al. 2012; Emery et al. 2016). Contrary to EPA, the ARA proportions in brain membranes increased with age and were higher in Loire compared to Seine. Both EPA and ARA are precursors in the production of eicosanoids (prostaglandins, leukotrienes and thromboxanes) (Gómez-Abellán and Sepulcre 2016). Leukotrienes play vital roles in the immune response of vertebrates, and can be produced by every tissue (Rowley et al. 1995; Sargent et al. 1999). Prostaglandins are of physiological importance for respiratory functions (McKenzie et al. 1998) and osmoregulation (Ruggeri and Thoroughgood 1985). ARA has also

been proven to reduce stress in fish through the modulation of cortisol, a hormone linked to stress regulation and modulated by prostaglandins (Koven et al. 2003; Van Anholt et al. 2004). Lebigre et al., (2022) analyzed the cortisol content in the scales of juvenile European sea bass from the same cohorts (including G1, G2 and G3) in both Seine and Loire estuaries. They found a peak of cortisol in 2019, the year in which the fish were sampled in the present study, compared to other years (2017 and 2018). They also reported that cortisol concentration increased with the ontogenetic stage. The authors underlined the fact that chronic stress has a negative effect on the growth of the fish. Then, the lower values of weight and size reported in fish from the Seine estuary could partly be explained by a higher chronic stress may be due to higher pollution level or strong salinity changes for example. All together, these results suggest that increasing ARA content in the brain with life stages could be induced by the selective retention of this FA to produce eicosanoids and cope with environmental and anthropogenic stressors.

6. Conclusions

The present study fills a gap of knowledge, reporting the variability in FA composition of wild European sea bass and evidencing molecular regulation of LC PUFA biosynthesis pathways across ontogenetic stages and in two main estuaries in Europe. We clearly observed a tissue-specific response of the distribution of FAs in both PL and NL with the brain having a specific FA composition compared to muscle and liver. Both habitat and ontogenetic stage had an influence on the general profile of membrane FA and on specific essential FA. These differences could be the consequence of a shift in prey selection as the fish grows, impacting the FA composition of the tissues with DHA prey proportions being higher for older fish. We found that activation of the LC PUFA synthesis pathway at the molecular level was associated with a lower level of DHA in the liver, although individuals did not appear to be able to compensate, as this was associated with a lower DHA proportion than that reported in dietary deficient sea bass juveniles reared experimentally and reported in the literature. This suggests a dietary LC PUFA deficiency in the natural environment that would not alter brain FA composition and thus function. The same individuals seemed to rather rely on EPA-rich prey. The higher ARA proportions in Loire compared to Seine could be related to eicosanoid

production necessary to regulate osmoregulation or stress response. These results clearly enhance the knowledge about the FA dynamics of euryhaline fish during the crucial juvenile life stage. Even though the lower proportions of DHA in membrane lipids compared to aquaculture fish fed with a standard diet cannot be described as a deficiency with certainty, it could be detrimental to young individuals in the context of a changing environment. It is then necessary to further investigate the effect of DHA depletion on the young stage of juvenile sea bass to study potential metabolic or behavioral implications in a potentially depleted trophic environment.

Competing interests

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

CRediT authorship contribution statement

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

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Supplementary material – Chapter 1

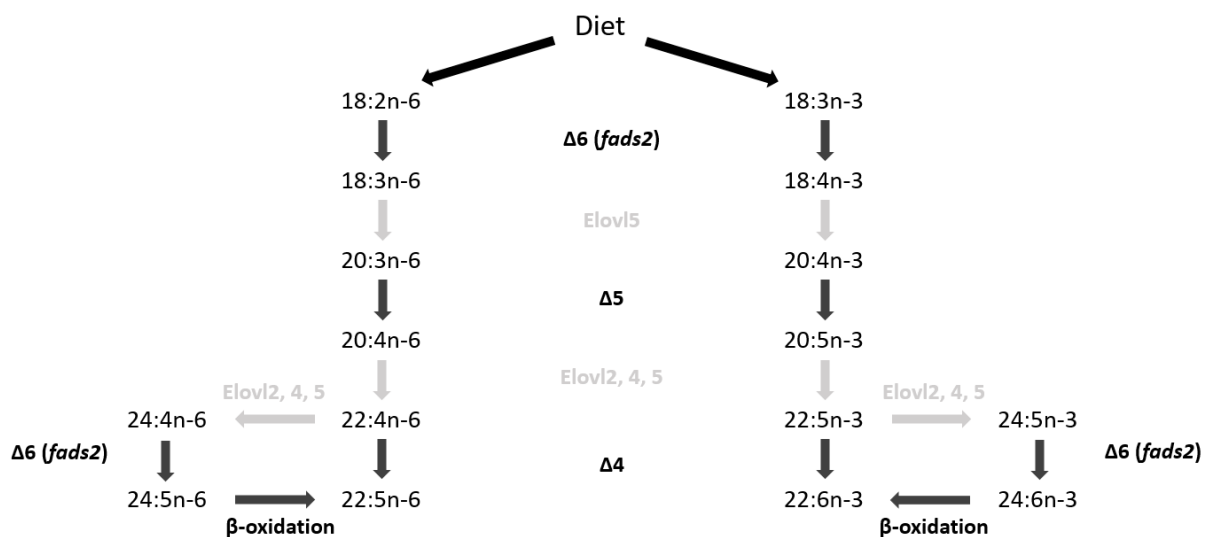


Figure S1: Biosynthetic pathways of LC PUFA from the precursors linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3) in teleosts. Black and gray arrows show reactions catalyzed by fatty acyl desaturase (“ Δx ”) and fatty acyl elongase (“*Elovl*”) activities, respectively. Modified from Garrido et al., (2019).

Chapter 1 - Spatial and ontogenetic modulation of fatty acid composition in juvenile European sea bass (*Dicentrarchus labrax*) from two French estuaries

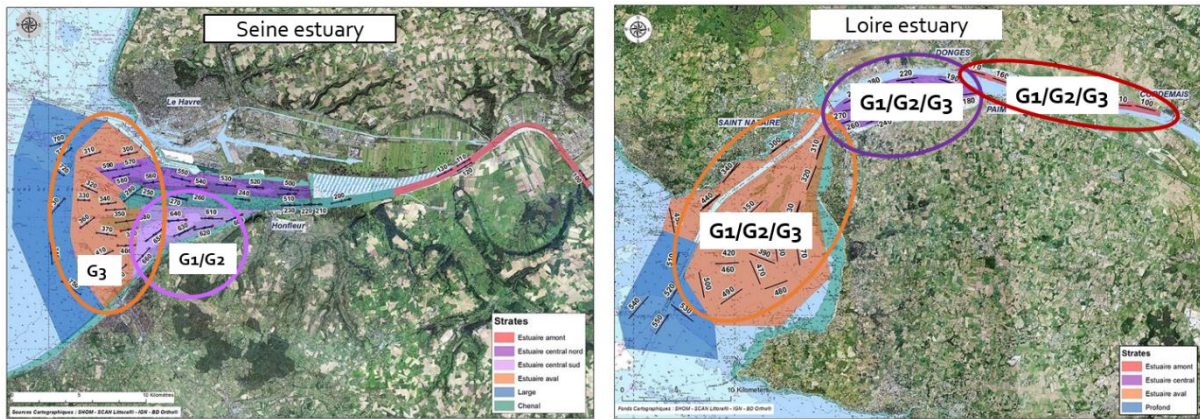


Figure S2: Sampling zones in estuaries of Seine and Loire. The colored ellipses represent the different layers of the estuaries. Orange ellipses represent the downstream of the estuary, purple and pink ellipses represent the center of the estuary and red ellipses represent the upstream of the estuary. The different life stages sampled at these locations are indicated in the ellipses.

Chapter 1 - Spatial and ontogenetic modulation of fatty acid composition in juvenile European sea bass (*Dicentrarchus labrax*) from two French estuaries

Table S1: GENBANK accession numbers and specific primers of the sequences used for each gene. *fads2*: Fatty acid desaturase 2; *lpl*: Lipoprotein lipase; *plag12b*: Group XIIB secretory phospholipase A2; *scd1b*: Stearoyl-CoA desaturase 1b; *sdhc*: Succinate dehydrogenase cytochrome b560 subunit; *ef1*: Elongation factor 1-alpha; *actin*: Beta Actin; *l13a*: Ribosomal protein L13a.

Gene	Forward primer (5'->3')	Reverse primer (5'->3')	Accession number
<i>fads2</i>	CCGCCGTGACTGGGTGGAT	GCACAGGTAGCGAAGGTAGTAAGACATAGA	EU647692
<i>lpl</i>	CAATGTGATCGTGGTGGACTG	CGTCGGGTAGTGCTGGTT	AM411614
<i>plag12b</i>	TTGAGTCAGTTGGCGGCTACTT	CGGTATTGGCACACTCCATCAC	KF857301
<i>scd1b</i>	GCTTGTGGCATACTTCATCCCTGGACTC	GGTGGCGTTGAGCATCACGGTGTA	FN868643
<i>sdhc</i>	ACATGGGCAAGGGCTTCAAA	CGATGATGGACAGACCGATAACG	KF857305
<i>ef1α</i>	CTGGAGGGCAGTGAAAAGAT	CATCAAGAGCCTCCAGCAGT	AJ866727
<i>act</i>	GTGCGTGACATCAAGGAGAA	AGACGCACAATCTTGAGAGCAG	AY148350
<i>l13a</i>	GCTTCGAGGAAATCACCAAG	CAACCTTCCATCCCTTGAAC	DT044539

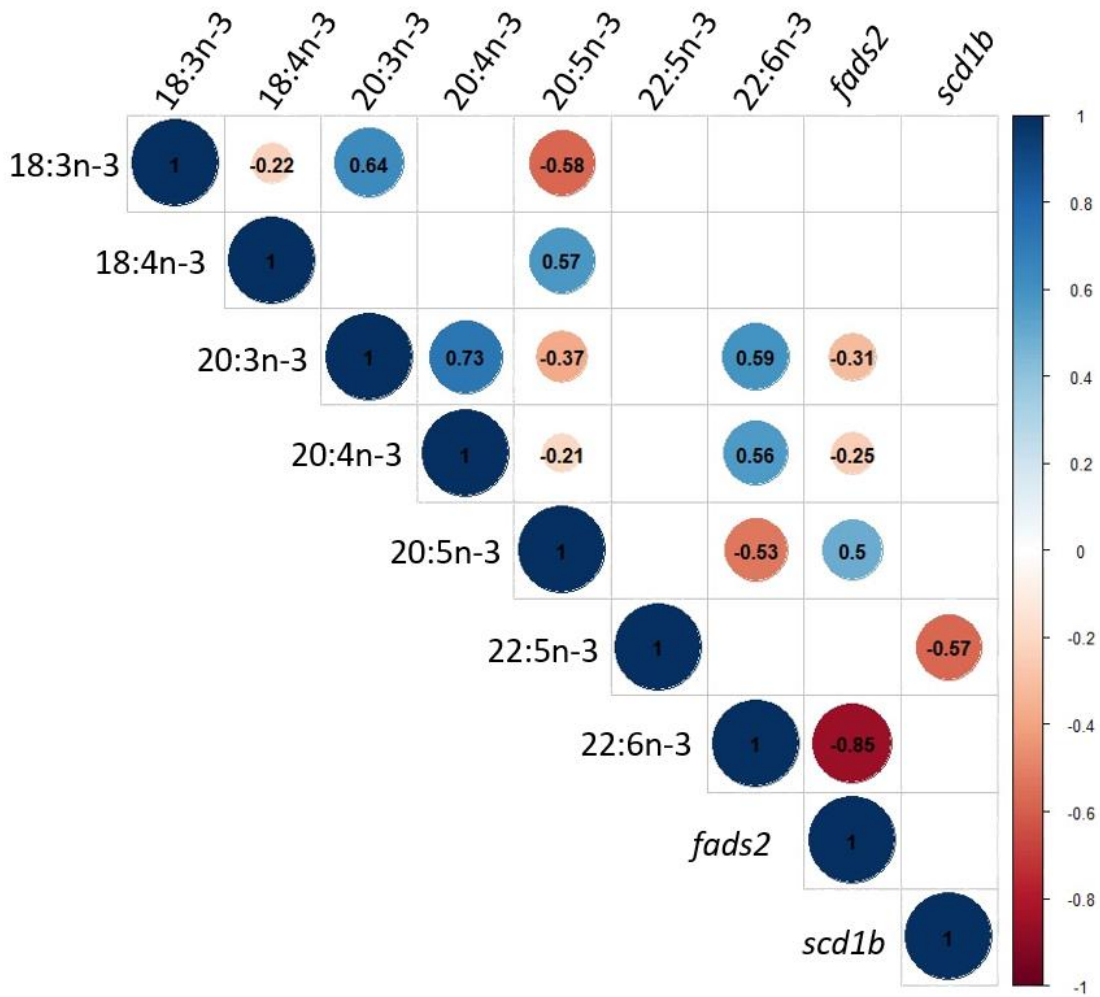


Figure S3: Correlations between the n-3 FA (percentage of total FA), fads2 and scd1b gene expression for the Seine G1 group (n=12). The colored circle represents a significant correlation between two values ($p < 0.05$). The numbers inside the circles are the associated Spearman's correlation.

Table S2: Fatty acid composition of polar (PL) and neutral (NL) fractions (Mean \pm SEM, percentage of total FA) in the liver, muscle and brain of European sea bass juveniles *Dicentrarchus labrax*. Letters indicate significant differences between the organs. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, - NS (not significant). SFA : Saturated Fatty Acid, MUFA: Monounsaturated Fatty Acid, PUFA: Polyunsaturated Fatty Acid, LC PUFA, long chain polyunsaturated fatty acid ≥ 20 C. Liver, n = 75, Muscle, n=50, Brain, n=49.

PL Fatty Acid	Liver	Muscle	Brain	Statistics
14 :0	0.6 \pm 0.01 ^c	0.5 \pm 0.01 ^b	0.3 \pm 0.01 ^a	***
16 :0	18.7 \pm 0.16 ^b	19.4 \pm 0.13 ^c	17.4 \pm 0.15 ^a	***
18 :0	8.8 \pm 0.1 ^b	6.45 \pm 0.05 ^a	9.9 \pm 0.11 ^c	***
24 :0	0.1 \pm 0.01 ^a	0.1 \pm 0.01 ^a	1.83 \pm 0.05 ^b	***
Σ SFA	29.6 \pm 0.21^b	27.7 \pm 0.15^a	30.1 \pm 0.21^b	***
16 :1n-7	1.7 \pm 0.04 ^a	1.7 \pm 0.06 ^a	2.2 \pm 0.04 ^b	***
16 :1n-9	0.6 \pm 0.01 ^b	0.5 \pm 0.01 ^a	1.3 \pm 0.02 ^c	***
18 :1n-7	2.4 \pm 0.03 ^b	2.5 \pm 0.04 ^b	1.5 \pm 0.01 ^a	***
18 :1n-9	7.0 \pm 0.23 ^a	7.4 \pm 0.10 ^a	18.6 \pm 0.22 ^b	***
20 :1n-7	0.2 \pm 0.01 ^b	0.2 \pm 0.01 ^b	0.1 \pm 0.01 ^a	***
20 :1n-9	0.4 \pm 0.01 ^a	0.5 \pm 0.01 ^a	1.1 \pm 0.02 ^b	***
20 :1n-11	0.1 \pm 0.01 ^a	0.1 \pm 0.01 ^b	0.1 \pm 0.01 ^c	***
24 :1n-9	1.1 \pm 0.04 ^b	0.67 \pm 0.03 ^a	4.2 \pm 0.14 ^c	***
Σ MUFA	14.5 \pm 0.24^a	14.4 \pm 0.16^a	29.6 \pm 0.41^b	***
20 :4n-6	5.9 \pm 0.07 ^c	4.1 \pm 0.07 ^b	3.1 \pm 0.07 ^a	***
20 :5n-3	10.3 \pm 0.19 ^b	13.6 \pm 0.27 ^c	3.4 \pm 0.07 ^a	***
22 :5n-3	2.0 \pm 0.07 ^b	2.7 \pm 0.10 ^c	1.0 \pm 0.03 ^a	***
22 :6n-3	31.0 \pm 0.46 ^c	29.0 \pm 0.54 ^b	22.3 \pm 0.30 ^a	***
Σ LC PUFA	50.8 \pm 0.36^b	51.7 \pm 0.29^b	31.0 \pm 0.47^a	***
Σ DMA	0.8 \pm 0.02^a	2.2 \pm 0.04^b	5.9 \pm 0.17^c	***

NL Fatty Acid	Liver	Muscle	Statistics
14 :0	1.7 ± 0.06	2.0 ± 0.05	***
16 :0	20.0 ± 0.53	19.1 ± 0.21	-
18 :0	4.7 ± 0.12	6.3 ± 0.18	***
24 :0	0	0.1 ± 0.02	***
Σ SFA	28.0 ± 0.60	29.9 ± 0.32	***
16 :1n-7	6.6 ± 0.23	6.1 ± 0.20	*
16 :1n-9	1.1 ± 0.05	0.6 ± 0.02	***
18 :1n-7	4.6 ± 0.09	4.4 ± 0.07	*
18 :1n-9	23.8 ± 1.09	15.3 ± 0.47	***
20 :1n-7	0.7 ± 0.05	1.1 ± 0.06	***
20 :1n-9	0.9 ± 0.06	1.3 ± 0.03	***
20 :1n-11	0.5 ± 0.04	1.2 ± 0.08	***
24 :1n-9	0.2 ± 0.03	0.6 ± 0.05	***
Σ MUFA	39.5 ± 1.13	32.48 ± 0.53	***
20 :4n-6	1.5 ± 0.08	1.5 ± 0.04	-
20 :5n-3	8.8 ± 0.43	9.6 ± 0.25	-
22 :5n-3	2.3 ± 0.14	2.9 ± 0.09	***
22 :6n-3	10.5 ± 0.72	11.0 ± 0.37	-
Σ LC PUFA	25.0 ± 1.40	27.9 ± 0.45	-

Table S3: PERMANOVA (euclidean method) results table for Liver, Muscle and Brain PL Fatty acids. Df: degrees of freedom. Data are based on 999 permutations. Significance was accepted at $p < 0.05$ and significant p-values are indicated in bold.

Liver	Df	Sum of squares	F-value	p-value
Estuary	1	66.32	3.29	0.051
Stage	2	50.05	1.24	0.272
Estuary x Stage	2	435.65	10.80	0.001
Residuals	69	1390.79		
Total	74	1942.80		

Muscle	Df	Sum of squares	F-value	p-value
Estuary	1	30.40	2.70	0.091
Stage	2	399.84	17.74	0.001
Estuary x Stage	2	108.12	4.80	0.006
Residuals	44	495.87		
Total	49	1034.23		

Brain	Df	Sum of squares	F-value	p-value
estuary	1	118.69	16.14	0.001
Stage	2	25.93	1.76	0.166
Estuary x Stage	2	58.73	3.99	0.015
Residuals	43	316.27		
Total	48	519.62		

Table S4: Results of pairwise comparisons of the PERMANOVA (Euclidean method) of PL FA profiles in liver, muscle and brain between the different groups. P-values were adjusted following the holm method. * $p < 0.05$, - NS (not significant).

	Liver	Muscle	Brain
Loire G1 – Loire G2	-	*	-
Loire G1 – Loire G3	*	-	-
Loire G1 – Seine G1	*	*	-
Loire G1 – Seine G2	-	-	*
Loire G1 – Seine G3	-	*	-
Loire G2 – Loire G3	-	-	-
Loire G2 – Seine G1	-	*	-
Loire G2 – Seine G2	-	-	*
Loire G2 – Seine G3	*	-	-
Loire G3 – Seine G1	-	*	-
Loire G3 – Seine G2	*	-	-
Loire G3 – Seine G3	*	-	-
Seine G1 – Seine G2	-	*	-
Seine G1 – Seine G3	*	*	-
Seine G2 – Seine G3	-	-	-

Résumé du Chapitre 1 en français

L'étude du chapitre 1 cherchait à comprendre l'influence du stade ontogénique et de l'estuaire d'origine sur la composition en acides gras (AG) du bar Européen juvénile. Cette étude voulait aussi caractériser les capacités de biosynthèse des AG de cette espèce dans le milieu naturel. Notamment, la composition en AG polyinsaturés à longue chaîne (AGPI LC) (ARA, EPA et DHA) a été étudiée. Pour ce faire, des juvéniles de bar ont été capturés en 2019 en estuaire de Seine et de Loire. Les AG de trois organes majeurs (foie, muscle et cerveau) ont été analysés. Premièrement, une distribution spécifique des AG en fonction des organes a été mise en évidence. Le cerveau s'est particulièrement démarqué par sa composition en AGPI LC, notamment par de faibles niveaux d'EPA par rapport au foie et au muscle. Cette distribution suggère des besoins différents en termes d'AG dans les tissus du fait de leurs différents rôles dans le métabolisme. Nous avons mis en évidence une distribution des AG influencée par l'interaction du stade ontogénique et de l'estuaire de vie des individus. Cette distribution peut être attribuée à plusieurs facteurs. Les besoins en AGPI des poissons évoluent au fil des années, modifiant ainsi la composition en AG entre les individus. De plus, quand le bar Européen grandit, son régime passe de proies benthiques, principalement composé de crustacés, à un régime piscivore. La composition en AG de ces proies est différente avec les poissons contenant plus d'AGPI LC, augmentant ainsi leurs proportions chez les stades plus âgés. Une production primaire différente entre les deux systèmes se traduit par une modification au bas de la chaîne trophique induisant des différences dans la présence des communautés de proies du bar Européen. Tous ces facteurs entre mêlés peuvent ainsi *in fine* modifier la composition en AG des poissons. Nous avons également mis en évidence une plus faible proportion de DHA dans les membranes du muscle et du Foie des SeineG1. Cela s'est traduit par une augmentation de l'expression de *fads2*, impliqué dans la biosynthèse des AGPI LC dans le foie. Dans un contexte de réchauffement climatique et de réduction de la production d'AGPI LC et particulièrement de DHA, ces niveaux plus bas de DHA pourraient être encore plus diminués pouvant être liés à des effets néfastes sur la physiologie et le comportement de bars dans le milieu naturel.

***Chapter 2 - Severe DHA
depletion did not affect the
swimming and metabolic
performance, but lowered
growth of wild-caught European
sea bass***

Research paper n°2 entitled “Severe DHA depletion did not affect the swimming and metabolic performance, but lowered growth of wild-caught European sea bass”

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1. Abstract

This study investigated the impact of a diet specifically depleted in docosahexaenoic acid (DHA) on growth, tissue fatty acid composition, and physiological performance in European sea bass (*Dicentrarchus labrax*). Fish were fed either a control diet or a DHA-depleted diet for five months, subjected to metabolic rate measurements, and swimming tests. While fish on the DHA-depleted diet exhibited lower growth rates, their tissue DHA levels remained unexpectedly high, implicating selective retention or biosynthesis. However, the DHA levels in membranes were lower in the Depleted fish compared to the Control. Upregulation of genes associated with long-chain polyunsaturated fatty acid (LC PUFA) biosynthesis was observed, though it failed to fully compensate for dietary DHA deficiency. Interestingly, neither metabolic rates nor swimming performance differed between the groups. Significant inter-individual variability was found across all measured parameters. The study sheds new light on the complex relationship between dietary DHA intake and various physiological parameters in marine fish, and opens avenues for exploring the underlying mechanisms of nutrient prioritization and their impact on individual variability.

Key words: Swimming performance, DHA, Gene expression, Metabolic Rate, Wild fish

2. Introduction

Long chain polyunsaturated fatty acids (LC-PUFA), and particularly those from the omega-3 series (LC n-3 PUFA) are key components of cell membranes and determinant for their structure and function (Sargent et al. 2003). Among them, docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) are the main component of phospholipids constituting the cell membranes (polar lipids, PL). In consequence, these LC n-3 PUFA are involved in cell functioning, making them necessary for tissue integrity, that determine whole individual biological performances (Sargent et al. 2003; Calder 2012). In fish, they thus have been shown to be vital for a multitude of physiological processes, ranging from growth, locomotion, immunity, stress response or reproduction (Tocher, 2003). DHA in particular, has been the most studied FA for its proven benefits on vertebrate health (Sargent et al. 1999; Lauritzen et al. 2001). It plays a major structural role in brain cells, ensuring its development and functioning and having a crucial impact for behavioral functions (Tocher, 2003).

In the natural environment, LC n-3 PUFA are primarily produced by marine microalgae, and are subsequently transferred up to higher trophic levels (Gladyshev et al. 2009; Parrish 2013). Their availability at the base of the trophic system is critical for higher organisms, such as marine fish, which have a limited ability to synthesize these essential fatty acids *de novo* from metabolic precursors. This limited biosynthesis ability would be due to a deficiency at the enzymatic level (Sargent et al., 2003; Tocher, 2003). Although some research has shown that marine fish can increase their fatty acid desaturation processes at a molecular level when they experience a low concentration of omega-3 polyunsaturated fatty acids, they still cannot make up for this deficiency at the tissue level (Vagner et al. 2007a, 2009).

The modifications of physico-chemical parameters induced by global changes such as ocean acidification, rising water temperatures, or hypoxia have been shown to collectively alter the physiology of marine microalgae, and the structures of their communities (Hixson and Arts, 2016). These changes could favor the dominance of microalgae species less efficient in producing DHA and other n-3 fatty acids (Colombo et al., 2020; Guschina and Harwood, 2006; Hixson and Arts, 2016). Such shifts are susceptible to affect the food chain, potentially leading to a reduced availability of DHA for higher trophic levels, notably fish (Galloway and Winder,

2015; Hixson and Arts, 2016). This decline in the availability of essential fatty acids could disrupt physiological processes and individual performance, and cascade through marine ecosystems, ultimately affecting population dynamics and community structures (Poloczanska et al. 2016).

Metabolic rate (MR) is a key parameter to assess the amount of energy expended by the different physiological functions of an animal over a specific period of time (Sánchez López de Nava and Raja 2023). Standard metabolic rate (SMR) represents the minimum viable oxygen consumption needed to maintain vital functions (osmoregulation, ventilation, cerebral functioning), and is evaluated as the lowest oxygen consumption measured when an animal is in fasting, unstressed, resting, and non-reproductive conditions. Maximum metabolic rate (MMR) is the highest aerobic metabolism possible for an organism, and is evaluated as the highest oxygen consumption during an effort test. The difference between MMR and SMR represents the aerobic scope (AS, Fry, 1971), defined as the maximum aerobic capacity (i.e. amount of oxygen available) for an individual to perform its non-mandatory activities such as growth, reproduction, digestion or locomotion including swimming performance (Claireaux and Lefrançois 2007; Norin and Metcalfe 2019).

Swimming performance is essential for fish survival in their natural habitats, as it determines many vital biological functions, including foraging, predator avoidance, migration, and spawning activities (Videler and Wardle 1991; Plaut 2001). Efficient swimming capacity is particularly crucial for species living in dynamic marine environments characterized by strong currents and high predation pressures. The mechanics and efficiency of swimming are tightly coupled with metabolic functions, making metabolic rate a physiological proxy for assessing the bioenergetic demands of swimming fish (Brett 1964; Plaut 2001).

The influence of FA, specifically LC n-3 PUFA, on swimming and metabolic performance of various fish species is a critical area of study in the context of changing environments. Studies in this domain, however, are not only scarce, but also show contradictory results. Some authors reported a lower maximum aerobic swimming speed reached (called as U_{crit} , Brett, 1964) in fish fed high levels of LC n-3 PUFA. In particular, Atlantic salmon (*Salmo salar*) showed a decrease in U_{crit} when they were fed a diet rich in LC n-3 PUFA compared to those fed with a canola oil-enriched diet, lower in LC n-3 PUFA (McKenzie et al. 1998). Similar results were

observed in European sea bass fed high levels of LC n-3 PUFA compared to those fed canola-oil or palm-oil, poorer in LC n-3 PUFA (Chatelier et al. 2006). In addition, lower active MR and AS was observed in fish fed high levels of LC n-3 PUFA (Chatelier et al. 2006). These authors also found a positive correlation between U_{crit} and high levels of monounsaturated fatty acids (MUFA), and particularly oleic acid (OA; 18:1n-9) suggesting that it could be used as an energy substrate to fuel sustained swimming. On the other hand, Arctic charr (*Salvelinus alpinus*) fed high levels of LC n-3 PUFA were found to have higher U_{crit} values than those fed a diet with high contents of rapeseed and palm oil, poorer in LC n-3 PUFA (Pettersson et al. 2010). This was also found in Atlantic salmon with individuals fed high levels of LC n-3 PUFA having higher U_{crit} than those fed a diet enriched with poultry fat, poorer in LC n-3 PUFA (Wagner et al. 2004). Conversely, dietary LC n-3 PUFA content did not influence the U_{crit} of golden gray mullet (*Chelon auratus*) but did increase the metabolic cost, in terms of O_2 consumption, during the swimming challenge and reduced the recovery capacities (Vagner et al. 2014). Litz et al. (2017) reported a positive relationship between triacylglycerol (TAG) levels and U_{crit} in Chinook salmon (*Oncorhynchus tshawytscha*), emphasizing the potential role of different fatty acid types in swimming proficiency. Lastly, the ratio of LC n-3 PUFA to saturated fatty acids (SFA), as well as the ratio of arachidonic acid (ARA) to SFA (Wagner et al. 2004) were both found to be positively correlated with U_{crit} in Atlantic salmon, highlighting the complex relationship between dietary FA composition and swimming performance in fish.

The European sea bass is a coastal fish that spends its growing juvenile stage (3-4 years) in estuaries, and is commercially important in Europe (Pérez-Ruzafa and Marcos 2014). This species 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). European sea bass is then a keystone in trophic web functioning (Pickett and Pawson 1994; Pérez-Ruzafa and Marcos 2014). This euryhaline species is capable of colonizing the whole estuary from almost freshwater zones to the oceanic coastline, supporting a wide range of salinity and temperature (Barnabé 1990). Due to its active nature, swimming performance could potentially act as a proxy for the fitness of individuals in the natural environment.

Estuaries are highly variable environments (in terms of e.g. temperature, salinity, oxygen saturation, water current, pollutant concentration), that are in the grip of multiple stressors

that induce a diversity of primary producers. This thus induces a variability of FA availability for consumers (Pohl 1979; Robins et al. 2016). In addition, the FA availability between the different habitats of sea bass (*i.e.* estuarine or coastal) can display spatial and seasonal variability (Galois et al. 1996).

Only one study investigated the relationship between dietary FA and the metabolic and swimming performance in European sea bass (Chatelier et al. 2006). The authors highlighted a detrimental effect on growth rate due to a deficiency in LC n-3 PUFA). However, these findings were based on domesticated fish, which have potential genotypic differences associated with different performances (metabolic or/and swimming) compared to their wild counterparts (Beamish 1978; Albert et al. 2012). Furthermore, domestic fish used in this previous study did not meet low dietary LC-PUFA during their early life stages as wild fish may encounter. It has been shown that the feeding conditions encountered during the very early life stages (larval stage) may have long lasting influence on the metabolism. Indeed European sea bass larvae fed a low LC n-3 PUFA diet enhance their LC n-3 PUFA synthesis pathways during their juvenile stage (Vagner et al. 2007b). Moreover, the reduction in dietary DHA is believed to be more pronounced than for other LC n-3 PUFAs (Colombo et al., 2020; Hixson and Arts, 2016) and DHA alone has been demonstrated to fulfill the needs for LC n-3 PUFA (Trushenski et al. 2012). Therefore, understanding the link between dietary DHA, its biosynthesis and metabolic and swimming performance on wild-caught individuals of an ecologically relevant model is crucial in a context of changing environment.

In this context, the aim of this study was to test the effect of dietary DHA depletion on (i) swimming performance and associated metabolic rates of wild-caught European sea bass, using critical swimming speed (U_{crit}) step protocol (Brett 1964), and (ii) the expression of genes involved in FA desaturation pathways to assess whether fish can activate biosynthetic pathways at the molecular level to compensate for DHA depletion. This work will improve our understanding of the impacts of an under-documented changing nutritional environment expected with global change on wild-caught European sea bass.

3. Material and Methods

3.1. Ethical statement

All of the experiments were performed with respect to 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.

3.2. Fish maintenance

3.2.1. 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.

3.2.2. 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. For one month, fish 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. Fish were fed exclusively on commercial pellets for 36 days. After these 66 days, 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) in open circuit, exposed to a 12h light / 12h dark photoperiod cycle, and natural temperature. Before being transported to their tank, 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, Réseautomatique, 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 by their FA contents (**Table 9**). 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 (later called Control and Depleted,

respectively) were reared in triplicates (n= 51 fish for Control condition and n= 50 for Depleted condition). Fish were fed this diet once a day (0.6-2 % of total tank biomass, depending on water temperature: 14.5°C ± 0.14, min-max = 10.3°C-18.7°C) for a minimum of five months. Oxygen saturation in the tanks was daily controlled and never lower than 90%. Every month, to monitor their growth, 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 9: 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 ¹	20	20
Wheat gluten ²	50	50
Canola oil ³	3	5
Fish oil ⁴	7	0
<i>Nannochloropsis</i> spp. Oil ⁴	0	5
Starch ²	6	6
Vitamins ⁵	4	4
Minerals ⁶	2	2
Cellulose ⁷	5	5
Betaine ⁸	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). ¹ LT70 Sopropêche, ² Roquette, Lestrem, France, ³ Oléandes, Mugron, France. ⁴ Polaris, Quimper, France. ⁵ Vitamin premix (IU or mg kg⁻¹ diet): DL- α 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). ⁶ 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).

3.3. Swimming challenge

3.3.1. Experimental setup

Swimming and metabolic performances were acquired on n=18 fish per experimental condition, using two swimming 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) is composed of a swim chamber (40Lx10Hx10W cm) and a hydraulic system that permits a laminar flow thanks to a honeycomb structure placed upstream of the swim chamber. The flow is generated using an external motor with a propeller. Its speed was controlled by the experimentator. Both motors were calibrated using a current meter before the experiments. In both tunnels, a flush pump was used to maintain the oxygen level 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 swimming chamber that were displayed live on a computer. Oxygen levels were measured using oxygen probes (FireSting O₂, 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).

3.3.2. Swimming protocol

Before each experiment, two fish (one per experimental condition) were electronically identified, isolated from their rearing tanks to be starved for a minimum of 48h. After 48h, i.e. the day before the swimming test, after 4:00 pm, fish were anesthetized with MS-222, measured in the bucket to further adjust the water speed in body length per second (BL s⁻¹), and transferred into a plastic bag (without exposing gills to the air) from the indoor acclimatizing tank to the swim chamber of the swimming respirometer. 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 swimming tunnel at a low velocity (0.5 BL s⁻¹). During all night, oxygen consumption and temperature were recorded and oxygen levels in the swim chamber were maintained thanks to the flush pump with cycles of 15 min of measurement and 5 min of flush. The following morning, a step protocol experiment (Brett, 1964, [Figure 30](#)) was conducted to measure swimming performance. Swimming performance can be assessed using an effort test such as a swimming challenge that aims to measure the critical swimming speed (U_{crit} ; the maximum velocity a fish can swim) (Brett 1964). Critical swimming speed (U_{crit}), and the associated oxygen consumption were measured. 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.

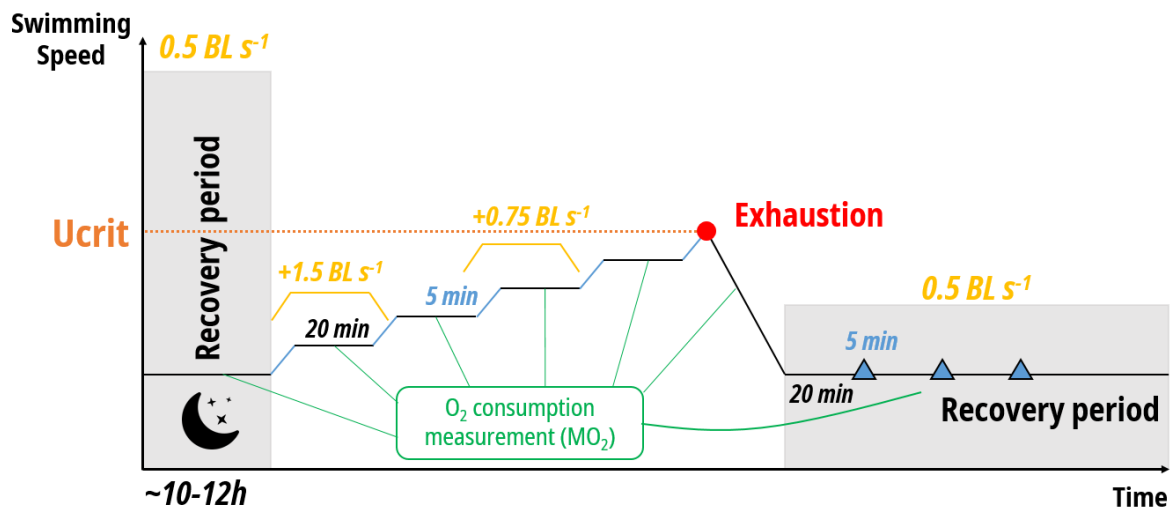


Figure 30: Schematic representation of the critical swimming protocol. The y axis represents the time and the x axis represents the swimming speed. Oxygen consumption measurements (MO_2) are indicated with the green lines. Swimming speed during the recovery periods (gray zones) was 0.5 BL s^{-1} and was increased two times by 1.5 BL s^{-1} and then by 0.75 BL s^{-1} every 20 min until the fish reached exhaustion. These 20 min measurements were separated by periods of 5 min (blue lines and triangles), where the oxygen levels were brought to saturation using flush pumps. EPOC: Excess Post Oxygen Consumption.

At each swimming step of the protocol (20 min), oxygen level was measured. During the velocity increase steps (5 min), the flushing pump allowed the restoration of oxygen levels that were never under 75%. Fish were considered exhausted when they failed to swim off the grid for 15s after light stimulation. When exhaustion was reached, the velocity of the current was immediately decreased to the recovery speed ($0.5 \text{ BL}\cdot\text{s}^{-1}$). 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 needed to restore: the tissue and cell storage of oxygen, the balance levels of metabolites 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 their white muscle (sampled on the left side, just before 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 microbial respiration was measured for 1h in the tunnels without fish. At the end of each experiment, the swim tunnel was fully cleaned with bleach tabs and freshwater. After thorough rinsing with fresh water, the tunnels were filled again with UV-treated sea water. During the swimming challenge, three fish died overnight while in the tunnel. By the conclusion of the experiment, the Control condition had 16 fish remaining, and the Depleted condition had 17 fish remaining.

3.4. Calculation of variables

3.4.1. Growth performance

For each individual, the Thermal Growth Coefficient (TGC; g degree day⁻¹) was calculated over the 5 months experimental period using the following **equation (1)**:

$$TGC = 1000 \times \left(\frac{W_{final}^{\frac{1}{3}} - W_{initial}^{\frac{1}{3}}}{T \times D} \right) \quad (1)$$

Where W_{final} is the weight at the end of the experiment, $W_{initial}$ is the weight at the beginning of the experiment, T is the average temperature during the experiment (14.5-15.1°C) 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) was also calculated over the 5 months of conditioning using the following **equation (2)**:

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

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

3.4.2. Critical swimming speed

U_{crit} ($BL \cdot s^{-1}$) 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} ($BL \cdot s^{-1}$) 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 ($BL \cdot s^{-1}$) is the increment of velocity before exhaustion of the fish (i.e. 1.5 or $0.75 \cdot BL \cdot s^{-1}$).

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.

3.4.3. Oxygen consumption

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

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

Where $\Delta[\text{O}_2]$ ($\text{mg O}_2 \text{ L}^{-1}$) 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).

$\Delta[\text{O}_2]/\Delta t$ is obtained by plotting $[\text{O}_2]$ 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 $\Delta[\text{O}_2]/\Delta t$ *bact*, which is the mean of these two measurements, was subtracted from the MO_2 measured with fish.

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

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

Where $m_{\text{corrected}}$ is the standard mass (100 g), MO_2 is the calculated oxygen consumption obtained with the **equation (4)**, and m is the fish weight (kg). 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).

3.4.4. Metabolic parameters

Standard Metabolic rate (SMR; $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was estimated by extrapolating the following **equation (6)** to a null swimming velocity (Brett 1964):

$$M_{O_2} = \text{SMR} e b^U \quad (6)$$

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

Maximal Metabolic Rate (MMR; $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was estimated by taking the highest value of MO_2 during the swimming challenge (Norin and Clark 2016).

Aerobic scope (AS; $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was then calculated with the following **equation (7)** (Lefrançois and Claireaux 2003):

$$AS = MMR - SMR \tag{7}$$

EPOC ($\text{mg O}_2 \text{ kg}^{-1}$) 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; $\text{mg O}_2 \text{ kg}^{-1} \text{ m}^{-1}$) was calculated as in (Clark et al. 2011) following **equation (8)**:

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

Where U_{crit} is expressed in m min^{-1} .

3.5. Lipid analysis

Fatty acid composition of liver, muscle and brain of all individuals tested in the swim tunnel ($n= 18$ per experimental condition) was measured. For each individual, lipids were extracted by grounding flash frozen tissues into a homogeneous powder in liquid nitrogen, and by diving ~ 50 g of each tissue into a 6 mL mixture of $\text{CHCl}_3/\text{MeOH}$ (2:1, v/v; Folch, 1957). The rest of the grounded liver was preserved at -80°C for further gene expression measurement (see 3.6).

3.5.1. Lipid separation

For each tissue, extracted lipids were separated into neutral lipid (NL) and polar lipid (PL) fractions following the method described by Le Grand et al. (2014), and using an aliquot (½) 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% H₂O (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).

3.5.2. Neutral and polar lipid transesterification

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 H₂SO₄/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.

3.5.3. FAME analysis

FAMES were analyzed in a Varian CP8400 gas chromatograph (Varian Inc., CA, USA) (GC) coupled with a flame-ionization detector (FID) as described by Mathieu-Resuge et al. (2019). FAMES were injected in splitless-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 calculating 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.

3.6. 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 relative expression levels of following transcripts were investigated (**Table S5**): Fatty acid desaturase 2 (*fads2*), Lipoprotein lipase (*lpl*), Stearoyl-CoA desaturase 1b (*scd1b*), fatty acid elongase 6 (*elovl6*) and fatty acid synthase (*fas*). These genes were chosen because they play a role in LC-PUFA or lipoprotein metabolism Rimoldi et al. (2016). The relative quantities of these transcripts of interest and those of three housekeeping genes (**Table S5**) (elongation factor 1-alpha, *ef1*; Beta Actin, *actin* and Ribosomal protein L13a, *l13a*) were determined by qPCR using a CFX96 Touch Real-Time PCR Detection system (Bio-Rad Laboratories Inc.). The relative quantities of transcripts were normalized with the $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001) using *ef1*, *actin* and *l13a* as reference genes since they met the stability requirement of the CFX Maestro software. This software also chooses a control sample automatically.

3.7. Statistical analysis

All analyses were performed using RStudio (V4.2.1). Growth performance, FA profiles, metabolic variables, U_{crit} and gene expression were compared between the Control and the Depleted groups using one-way ANOVA. For all groups, one outlier was removed from the data-set. For SMR and EPOC, one individual was removed as it was considered as an outlier. The normality and homoscedasticity of residuals was tested using a Shapiro-Wilk and Levene test, respectively. When the assumptions of the ANOVA were not met, a non parametric Kruskal-Wallis test was used instead. The correlation between DHA proportions between performance, lipid composition or gene expression was tested using Spearman's correlation. Significativity was accepted at $p < 0.05$.

4. Results

4.1. Growth performance

The results about growth performance are summarized in [Table 10](#). The TGC was significantly lower in the Depleted groups compared to the Control groups ([Table 10](#)). Three individuals from the Depleted condition had negative TGC 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 ($p > 0.05$). The TGC of the depleted groups was negatively correlated with the DHA proportions in muscle, but not with those in liver or brain ([Figure S4](#)).

Table 10: Growth performance and condition factor of European seabass fed the Control diet (n=15) or the Depleted diet (n=16). The p-values of the one-way ANOVA or Kruskal-Wallis tests when the ANOVA conditions were not met are indicated. TGC: Thermal Growth Coefficient (g deg.day^{-1}), TL: Total Length (cm). Data are presented as mean \pm SEM

	Control groups	Depleted groups	p-value
TGC	0.26 \pm 0.02	0.13 \pm 0.04	0.009
Final Fulton's K	1.2 \pm 0.0	1.1 \pm 0.0	0.05
Final TL	21.1 \pm 0.3	20.8 \pm 0.4	0.58
Final Weight	114.9 \pm 5.6	105.9 \pm 6.9	0.32

4.2. Fatty acid composition

In PL of all tissues, the main FA families (SFA, MUFA, PUFA) showed no significant differences ($p>0.05$) between the dietary treatments ([Table 11](#), [Table 12](#), [Table 13](#)). Comparing the two dietary groups, less differences in FA composition were found in the brain (only two FA, including 24:1n-9 and DHA), than in the liver and muscle (six FA).

All tissues showed high levels of DHA in both NL and PL compared to the DHA contained in the diet.

Although there is only 0.5% of DHA in the Depleted diet, the three tissues contained DHA in the NL and PL, with the latter having higher proportions of DHA than in NL.

DHA proportions in PL were lower in all organs of the Depleted fish compared to Control fish. This difference in DHA was the strongest in the liver, with DHA being 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. 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 an EPA/DHA ratio twofold higher in the liver of the Depleted group compared to the Control group ([Table 11](#)).

The total n-3 proportions in PL did not follow the same trend as DHA, and it was only significantly lower 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 11](#), [Table 12](#), [Table 13](#)).

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

The DHA and EPA ratio between tissue and diet is shown in [Figure 31](#). If the value of this ratio is above 1, it suggests selective retention in the tissue. For both 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 31A](#)). 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 EPA tissue/diet ratio was above 1 in the liver and muscle but was lower than one in the brain (Figure 31B).

Table 11: Fatty acid profiles of Polar Lipids (% of total FA) in the liver of European sea bass fed the Control (n=15) and the DHA Depleted (n=16) diets. PL: polar lipids. Data are presented as mean \pm SEM. Statistical significance is indicated by the p-value (one-way ANOVA).

PL FA	Liver		Statistics p-value
	Control	Depleted	
16:0	16.5 \pm 0.4	15.7 \pm 0.4	0.13
18:0	10.8 \pm 0.4	11.2 \pm 0.4	0.44
24:0	0.1 \pm 0.0	0.2 \pm 0.0	0.04
Σ SFA	28.8 \pm 0.5	28.3 \pm 0.2	0.45
16 :1n-7	1.7 \pm 0.1	2.0 \pm 0.1	0.02
16 :1n-9	0.8 \pm 0.0	0.8 \pm 0.0	0.33
18 :1n-7	2.5 \pm 0.1	2.6 \pm 0.1	0.49
18 :1n-9	16.0 \pm 0.6	16.5 \pm 0.4	0.48
20 :1n-9	0.7 \pm 0.0	0.8 \pm 0.0	0.001
24 :1n-9	0.4 \pm 0.0	0.3 \pm 0.0	0.70
Σ MUFA	22.5 \pm 0.7	23.5 \pm 0.5	0.27
18:2n-6	4.7 \pm 0.1	5.1 \pm 0.3	0.22
18 :3n-3	0.7 \pm 0.0	0.8 \pm 0.1	0.11
20 :4n-6	3.7 \pm 0.1	5.4 \pm 0.1	<0.001
20 :5n-3	11.2 \pm 0.3	15.2 \pm 0.4	<0.001
22 :5n-3	1.6 \pm 0.1	1.5 \pm 0.1	0.09
22 :6n-3	22.8 \pm 0.4	16.4 \pm 0.7	<0.001
Σ PUFA	48.0 \pm 0.6	47.3 \pm 0.5	0.43
Σ LC-PUFA	40.5 \pm 0.7	39.3 \pm 0.7	0.22
Σ n-6	9.9 \pm 0.2	12.0 \pm 0.3	<0.001
Σ n-3	37.2 \pm 0.6	34.4 \pm 0.6	0.002
Σ DMA	0.4 \pm 0.0	0.5 \pm 0.1	0.11
EPA/DHA	0.5 \pm 0.0	1.0 \pm 0.1	<0.001

Table 12: Fatty acid profiles of Polar Lipids (% of total FA) in the muscle of European sea bass fed the Control (n=15) and the DHA Depleted (n=16) diets. PL: polar lipids Data are presented as mean \pm SEM. Statistical significance is indicated by the p-value (one-way ANOVA).

PL FA	Muscle		Statistics p-value
	Control	Depleted	
16:0	20.5 \pm 0.3	20.5 \pm 0.5	0.88
18:0	7.4 \pm 0.1	7.5 \pm 0.2	0.74
24:0	0.2 \pm 0.0	0.2 \pm 0.0	0.88
Σ SFA	29.0 \pm 0.4	29.2 \pm 0.3	0.62
16 :1n-7	1.3 \pm 0.1	1.8 \pm 0.1	0.002
16 :1n-9	0.4 \pm 0.0	0.5 \pm 0.0	0.05
18 :1n-7	2.3 \pm 0.0	2.2 \pm 0.1	0.17
18 :1n-9	13.9 \pm 0.2	14.1 \pm 0.5	0.58
20 :1n-9	0.6 \pm 0.0	0.6 \pm 0.0	0.17
24 :1n-9	0.1 \pm 0.0	0.1 \pm 0.0	0.99
Σ MUFA	19.5 \pm 0.6	19.1 \pm 0.3	0.66
18:2n-6	5.1 \pm 0.3	5.5 \pm 0.2	0.64
18 :3n-3	0.8 \pm 0.0	1.0 \pm 0.0	0.002
20 :4n-6	2.7 \pm 0.1	3.4 \pm 0.1	<0.001
20 :5n-3	14.3 \pm 0.3	15.8 \pm 0.7	0.04
22 :5n-3	2.5 \pm 0.0	2.0 \pm 0.1	<0.001
22 :6n-3	21.4 \pm 0.4	18.2 \pm 0.9	0.001
Σ PUFA	49.6 \pm 0.7	49.6 \pm 0.4	0.93
Σ LC-PUFA	41.4 \pm 0.8	41.4 \pm 0.7	0.98
Σ n-6	9.2 \pm 0.3	10.7 \pm 0.3	0.002
Σ n-3	39.3 \pm 0.7	38.3 \pm 0.7	0.32
Σ DMA	2.0 \pm 0.1	2.0 \pm 0.1	0.91
EPA/DHA	0.7 \pm 0.0	0.9 \pm 0.1	<0.001

Table 13: Fatty acid profiles of Polar Lipids (% of total FA) in the brain of European sea bass fed the Control (n=15) and the DHA Depleted (n=16) diets. PL: polar lipids. Data are presented as mean \pm SEM. Statistical significance is indicated by the p-value (one-way ANOVA).

PL FA	Brain		Statistics p-value
	Control	Depleted	
16:0	16.8 \pm 0.4	16.7 \pm 0.2	0.93
18:0	9.8 \pm 0.1	8.9 \pm 0.1	0.33
24:0	1.7 \pm 0.0	1.8 \pm 0.1	0.05
Σ SFA	28.7 \pm 0.3	28.5 \pm 0.4	0.72
16 :1n-7	2.4 \pm 0.0	2.5 \pm 0.0	0.07
16 :1n-9	1.5 \pm 0.0	1.5 \pm 0.0	0.33
18 :1n-7	1.5 \pm 0.0	1.5 \pm 0.0	0.87
18 :1n-9	19.3 \pm 0.2	19.9 \pm 0.2	0.11
20 :1n-9	1.3 \pm 0.0	1.4 \pm 0.0	0.10
24 :1n-9	4.5 \pm 0.1	5.0 \pm 0.2	0.01
Σ MUFA	31.8 \pm 0.3	32.3 \pm 0.5	0.37
18:2n-6	0.5 \pm 0.0	0.5 \pm 0.0	0.84
18 :3n-3	0.1 \pm 0.0	0.1 \pm 0.0	0.31
20 :4n-6	3.3 \pm 0.1	3.4 \pm 0.1	0.20
20 :5n-3	3.5 \pm 0.1	3.7 \pm 0.1	0.10
22 :5n-3	0.8 \pm 0.0	1.0 \pm 0.0	0.02
22 :6n-3	21.2 \pm 0.3	18.9 \pm 0.4	<0.001
Σ PUFA	31.3 \pm 0.2	30.5 \pm 0.5	0.14
Σ LC-PUFA	29.1 \pm 0.2	28.3 \pm 0.5	0.13
Σ n-6	4.8 \pm 0.1	5.2 \pm 0.2	0.08
Σ n-3	25.9 \pm 0.2	24.6 \pm 0.6	0.05
Σ DMA	7.5 \pm 0.2	8.2 \pm 0.3	0.08
EPA/DHA	0.17 \pm 0.0	0.2 \pm 0.0	0.02

Table 14: Fatty acid profiles of Neutral Lipids (% of total FA) in the liver, muscle and brain of European sea bass fed the Control (n=15) and the DHA Depleted (n=16) diets. NL: neutral lipids. Data are presented as mean \pm SEM. Statistical significance is indicated by the p-value (one-way ANOVA).

NL FA	Muscle		Statistics p-value
	Control	Depleted	
14:0	2.7 \pm 0.1	2.3 \pm 0.1	<0.001
16:0	19.0 \pm 0.2	18.9 \pm 0.2	0.71
18:0	4.2 \pm 0.1	3.9 \pm 0.1	0.14
Σ SFA	26.8 \pm 0.4	25.9 \pm 0.3	0.06
16 :1n-7	5.6 \pm 0.1	7.1 \pm 0.2	<0.001
18 :1n-7	3.2 \pm 0.0	3.1 \pm 0.1	0.15
18 :1n-9	30.3 \pm 0.3	32.8 \pm 0.3	<0.001
20 :1n-9	1.8 \pm 0.0	1.9 \pm 0.1	0.61
Σ MUFA	43.5 \pm 0.4	47.2 \pm 0.5	<0.001
18 :2n-6	9.3 \pm 0.2	9.3 \pm 0.4	0.97
18 :3n-3	1.6 \pm 0.0	1.8 \pm 0.1	0.03
20 :4n-6	0.5 \pm 0.0	0.7 \pm 0.0	<0.001
20 :5n-3	6.9 \pm 0.3	6.9 \pm 0.3	0.95
22 :5n-3	1.2 \pm 0.1	0.7 \pm 0.1	<0.001
22 :6n-3	4.45 \pm 0.2	2.8 \pm 0.2	<0.001
Σ PUFA	28.8 \pm 0.4	26.2 \pm 0.5	<0.001
Σ LC-PUFA	14.7 \pm 0.5	12.5 \pm 0.4	0.001
Σ n-6	11.3 \pm 0.2	11.1 \pm 0.7	0.81
Σ n-3	16.0 \pm 0.5	13.5 \pm 0.4	<0.001
Σ DMA	0.5 \pm 0.0	0.3 \pm 0.0	<0.001
EPA/DHA	1.5 \pm 0.1	2.6 \pm 0.2	<0.001

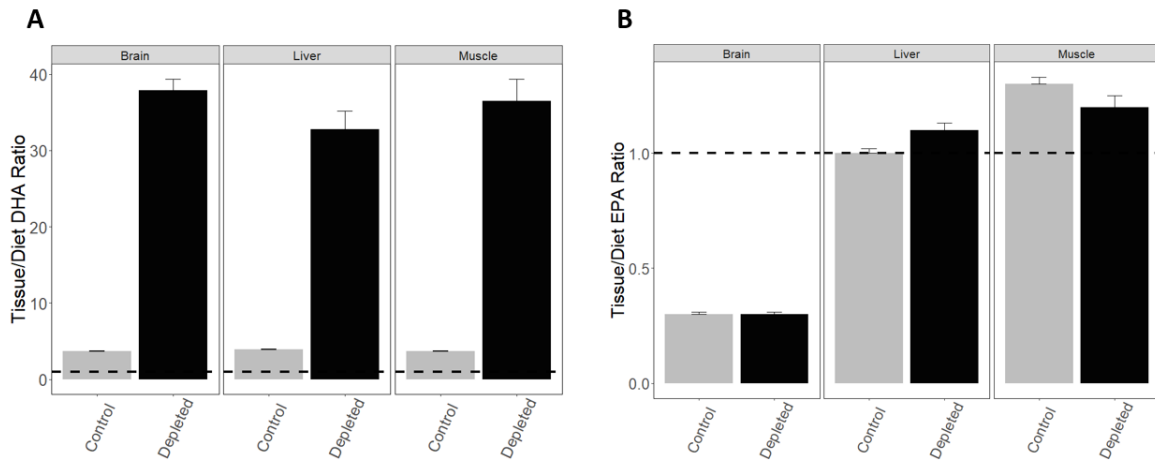


Figure 31: Tissue to diet ratio (\pm SEM) for DHA (A) and EPA (B). The gray bars represent the Control condition (n=15) and the black bars represent the Depleted condition (n=16). The horizontal dotted line represents the 1:1 ratio between the tissue and the diet.

4.3. Swimming and metabolic performance

The MO_2 increased exponentially with the swimming speed for both dietary treatments (**Figure 32**). No significant differences were observed between the two dietary groups. This was also the case for the metabolic variables (SMR, MMR, AS, EPOC and NCOT) and U_{crit} (results summarized in **Table 15**) that were not affected by the dietary treatment ($p > 0.05$).

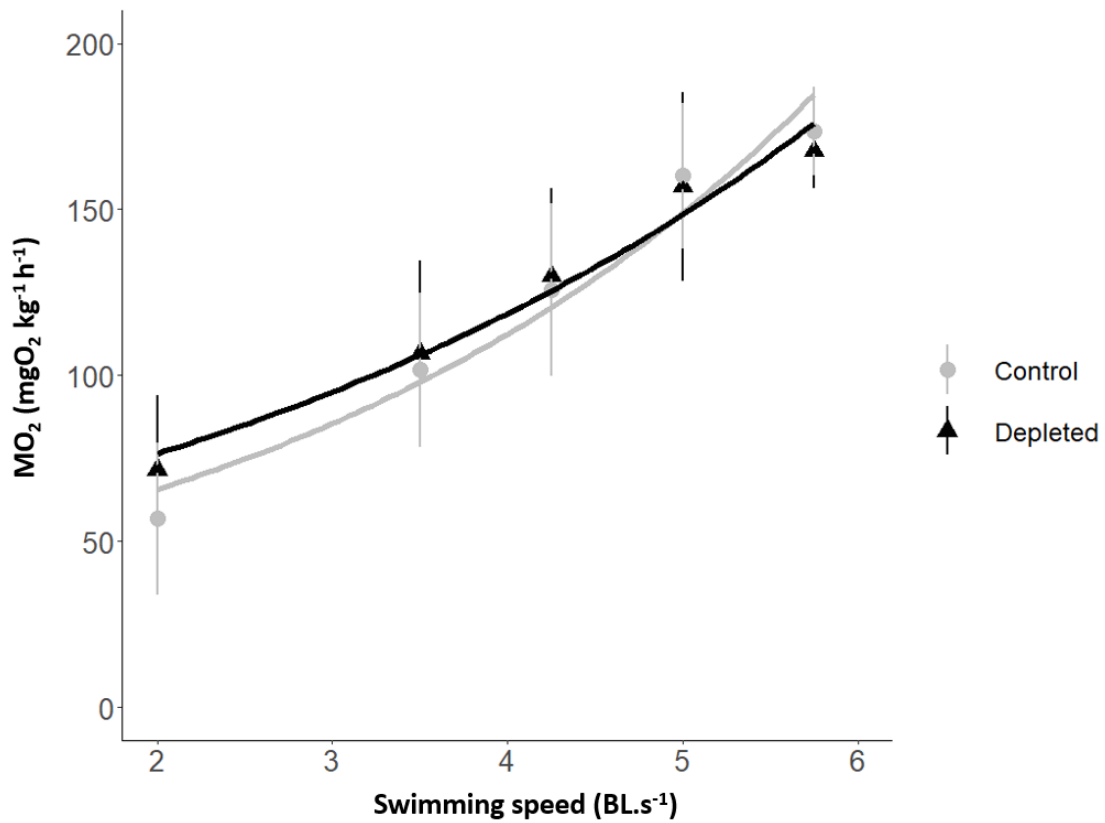


Figure 32: Oxygen consumption (MO₂, mg O₂ kg⁻¹ h⁻¹ ± SEM) as a function of swimming speed for both dietary groups. The Control group is represented by circles in grey (n=14) and the Depleted group is represented by the triangles in black (n=16). Both lines are exponential curves fitted on MO₂ as a function of swimming speed. (Control group curve: $y=32.833e^{0.3059x}$; $r^2 = 0.99$; Depleted group curve: $y=46.094e^{0.2356x}$; $r^2=0.99$).

Table 15: Metabolic and swimming performance for sea bass fed either the Control diet or the DHA Depleted diet. Data are standard metabolic rate (SMR, mg O₂ kg⁻¹ h⁻¹), maximum metabolic rate (MMR, mg O₂ kg⁻¹ h⁻¹), aerobic scope (AS, mg O₂ kg⁻¹ h⁻¹), excess post-oxygen consumption (EPOC, mg O₂ kg⁻¹) and critical swimming speed (U_{crit}, BL s⁻¹). Data are presented as mean ± SEM, n is the number of individuals statistically tested. The statistical difference is indicated by the p-value (one-way Anova or Kurskal-Wallis).

Dietary Condition	SMR	MMR	AS	EPOC	U _{crit}	NCOT
Control	26.8 ± 1.3 (n=15)	160.6 ± 8.1 (n=15)	133.8 ± 7.7 (n=15)	1489.9 ± 103.4 (n=14)	4.8 ± 0.2 (n=14)	2.3 ± 0.1 (n=15)
Depleted	25.3 ± 1.3 (n=15)	155.3 ± 6.7 (n=16)	128.9 ± 6.6 (n=15)	1463.4 ± 131.1 (n=16)	4.7 ± 0.1 (n=16)	2.2 ± 0.1 (n=16)
p-value	0.42	0.42	0.37	0.88	0.49	0.53

4.4. Gene expression

The relative expression of *fads2*, *fas* and *lpl* transcripts were significantly higher in the Depleted fish than in the Control fish (p<0.01, **Figure 33**). The relative gene expression of *elovl6* and *scd1b* measured in the liver were not significantly different between dietary groups (**Figure 33**).

Significant negative correlations were found between the hepatic DHA proportions and the relative expressions of *fads2*, *fas*, *lpl* and *scd1b* for the Depleted group (**Figure 34**).

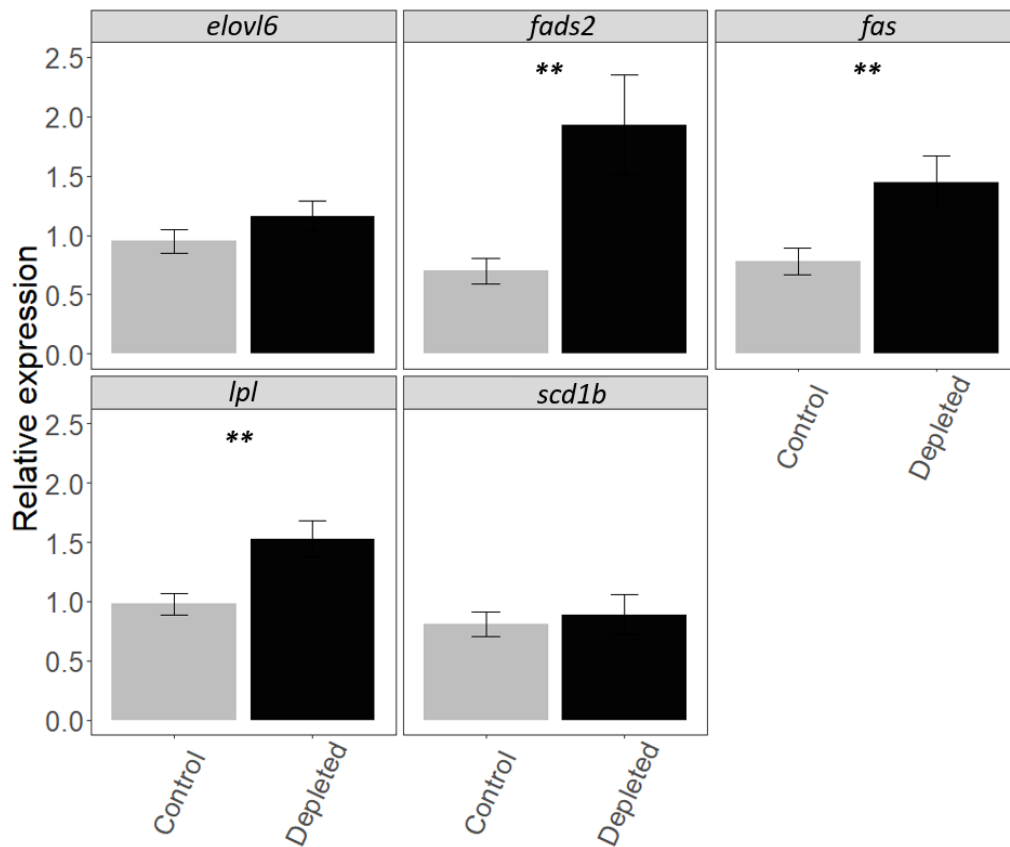


Figure 33: Relative expression of genes (\pm SEM) coding for enzymes involved in lipids metabolism in the liver of European sea bass fed the Control (n=16) or the DHA Depleted (n=14) diet. *elovl6* : fatty acid elongase 6, *fads2*: fatty acid desaturase 2, *fas*: fatty acid synthase, *lpl*: lipoprotein lipase, *scd1b*: stearyl-CoA desaturase. Asterisks represent statistical significance between conditions (one-way ANOVA or Kruskal-Wallis test). ** p<0.01.

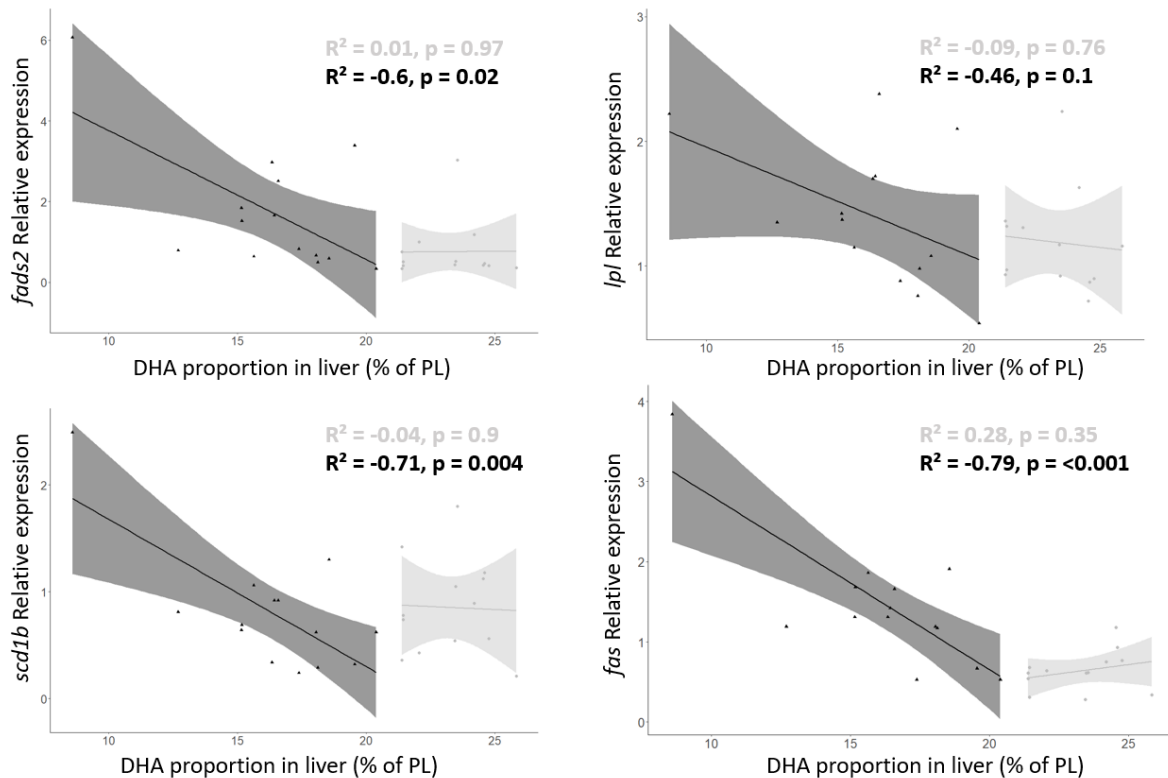


Figure 34: Linear regression between the PL DHA content in liver and the relative gene expressions of *fads2* (A), *fas* (B), *lpl* (C) and *scd1b* (D). The grey regression line represents the Control condition (n=16) and the black regression line represents the Depleted condition (n=14).

5. Discussion

We investigated the effects of a long-term (5 months) dietary DHA limitation on the growth and swimming performance as well as the metabolic rate of wild caught juvenile European sea bass. While the DHA proportions in the tissues of Depleted sea bass remained high, they were significantly reduced compared to the Control. The DHA limitation negatively affected growth but not swimming performance or metabolic rate. We highlighted high variations between individuals on all of the measured traits.

5.1. Fatty acid composition

Despite the very low proportions of DHA in the diet, high levels of DHA were found in the tissues of fish from the Depleted condition. This was highlighted by a high DHA tissue/diet ratio in all tissues suggesting selective retention or internal synthesis of DHA (Závorka et al. 2021). However, the DHA levels in all organs (muscle, liver and brain) of fish from the Depleted

condition remained significantly lower compared to those in the Control fish group. Interestingly, the tissue/diet DHA ratio was above one in the control diet suggesting that the Control fish also retained or synthesized DHA. On the other hand, EPA tissue/diet ratio showed a tissue specific response. The muscle and liver had a tissue/diet ratio equal or superior to one in both conditions but the brain had a ratio below 0.5. This suggests that EPA is not retained in the same way across the organs. This result could also mean that the EPA was used to synthesize DHA in the brain (Kainz et al. 2006) or catabolized, as it is the case in mammals (Chen and Bazinet 2015). We also observed higher proportions of EPA and ARA in the liver and muscle of fish from the Depleted condition. Considering their role as eicosanoids precursors, this might have implications about the fish stress response (Rowley et al. 1995). Along with the significant effect of the DHA depleted diet on DHA proportions, a high inter-individual variability was also observed in fish fed this diet. Indeed, there is notable inter-individual variability within each condition group in terms of tissue DHA proportions. This intra-group variability leads to surprising inter-group findings, such as some individuals from the Depleted condition having higher tissue DHA proportions than certain 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 membranes of individuals that could vary on a multitude of levels such as retention or synthesis. High individual variations were previously observed in golden gray mullets fed high (17.5 %) and low (1.2 %) levels of EPA+DHA (Salin et al. 2021).

5.2. Growth performance

The dietary DHA limitation was associated with a lower growth rate and induced a higher growth rate variability between individuals. Previous studies reported a negative effect of low LC n-3 PUFA diet (0.7% of dry mass [DM]) on TGC in European sea bass fed for a minimum of 129 days (Gourtay et al. 2018). A study on juvenile cobia (*Rachycentron canadum*) concluded that the requirements for this carnivorous fish could be met by DHA only (Trushenski et al. 2012). However, for most species, including European sea bass the LC n-3 PUFA requirements are expressed as EPA+DHA (Skalli and Robin 2004). DHA plays a key role in the growth of fish (Watanabe and Kiron 1994). Yet, for the fish from the Depleted condition, high DHA in muscle was associated with a lowered growth rate. The tissue/diet ratio was also negatively correlated with TGC. This paradoxical result was previously observed in other species and it

suggests a cost of the physiological adaptation to low DHA (Murray et al. 2014; Lazzarotto et al. 2015; Závorka et al. 2021). The hypothesis of a DHA synthesis in Depleted fish is supported by the upregulation of the expression of *fads2*, involved in LC n-3 PUFA metabolism observed in this group, as well as by the negative correlation between gene expression and hepatic DHA content. Notably, *fads2* codes for the delta6 desaturase, the first enzyme involved in the biosynthesis of LC n-3 PUFA and considered as a limiting step of (Vagner and Santigosa 2011). This upregulation could suggest an internal synthesis in the liver to maintain the DHA levels in the tissue, which would be energy costly. 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 activity (Geay et al., 2010). The lower DHA levels in the liver of Depleted fish compared to control fish could suggest either 1) that FADS2 activity and *fads2* expression are not necessarily related, or 2) 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 consistent regardless of dietary variations (Castro et al. 2016; Viegas et al. 2022).

5.3. Swimming and metabolic performance

Despite lower growth in the Depleted fish, potentially due to the energetic costs of maintaining high tissue DHA levels or considering the role of DHA in growth, we observed no corresponding increase in metabolic rates or maximum aerobic swimming performance. This disconnect suggests other mechanisms may be in play, masking or offsetting the anticipated energetic costs. It was previously observed on wild juvenile salmon that showed no differences in MMR between fish fed a low LC n-3 PUFA diet and fish having normal levels of dietary LC n-3 PUFA (Závorka et al. 2021). The authors suggested that, even though the deficiency decreased mitochondrial ATP production, mitochondrial metabolism could show a resilience to low levels of LC n-3 PUFA (Guderley et al. 2008; Martin et al. 2013; Závorka et al. 2021). In addition, as mentioned earlier, some individuals from the Depleted condition had DHA levels in tissues similar or higher to some Control individuals. This inter-individual variability could be an explanation for the fact that despite the deficiency, some individuals

may be able to physiologically adjust at the metabolic level. Moreover, the metabolic rate is an integrative trait that depends on multiple factors (ventilation, oxygen transport, mitochondrial performance) which could have been affected without it being directly visible on the metabolic rate. Individuals might also use different energy sources to fuel their needs; for example, individuals deficient in DHA might use MUFA to sustain the swimming (McKenzie 2001; Chatelier et al. 2006). While having a high level of EPA in the diet did not avoid the decrease in growth, having a high amount of EPA might have balanced out the differences in metabolic rate. This is because EPA has been suggested to influence metabolic rate at the cellular level (McKenzie 2001). In addition, Depleted fish might have retained the DHA from their previous diets (before being fed the experimental diet). Therefore, the five month conditioning might have been too short to induce detrimental effects on the metabolic and swimming performance of the Depleted fish.

6. Conclusions

This study is the first to explore the impact of a specific DHA dietary restriction, distinct from EPA, on the metabolic and swimming performance of marine fish. Despite a severe DHA dietary restriction, fish tissues displayed high DHA proportions suggesting selective retention or synthesis. Although there was an upregulation in the expression of genes associated with LC PUFA biosynthesis, it did not compensate for the DHA deficiency. One hypothesis is that the stimulation of the LC PUFA synthesis might be 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 rate or swimming performance. Another hypothesis is that the lower DHA content observed in the tissues of fish from the depleted group may be directly responsible for lower growth. Finally, considerable inter-individual variability was evidenced in all the measured variables (growth, metabolic rate, U_{crit}). Further research should try to investigate the energy trade-offs during a dietary DHA limitation.

Supplementary materials – Chapter 2

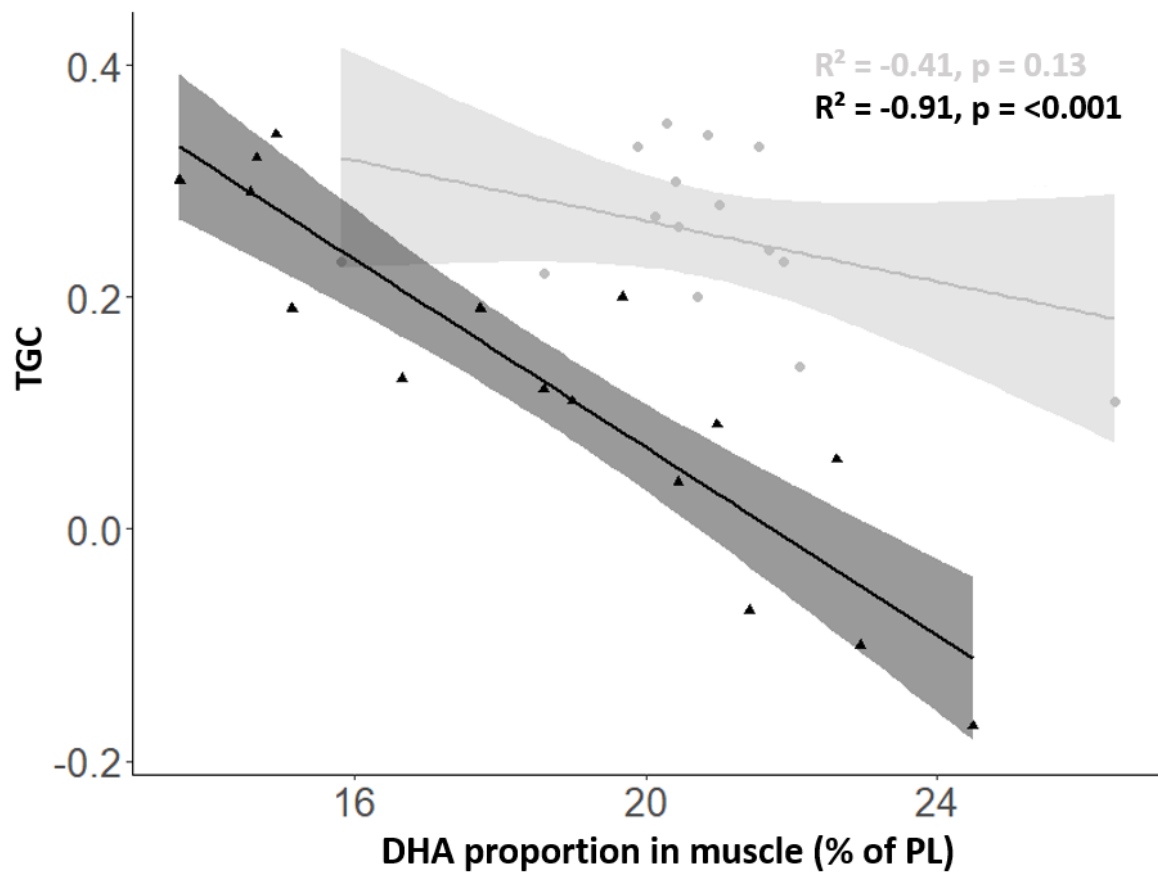


Figure S4 : Linear regression between Thermal Growth Coefficient (TGC) and DHA Muscle proportion. The gray regression line represents the Control condition (n=15) and the black regression line represents the Depleted condition (n=16).

Chapter 2 - Severe DHA depletion did not affect the swimming and metabolic performance, but lowered growth of wild-caught European sea bass

Table S5: GENBANK accession numbers and specific primers of the sequences used for each gene.

Genes	Forward primer	Reverse primer	Accession number
<i>fads2</i>	CCGCCGTGACTGGGTGGAT	GCACAGGTAGCGAAGGTAGTAAGACATAGA	EU647692
<i>lpl</i>	GATCGTGGTGGACTG	CGTCGGGTAGTGCTGGTT	AM411614
<i>scd1b</i>	GCTTGTGGCATACTTCATCCCTGGACTC	GGTGGCGTTGAGCATCACGGTGTA	FN868643
<i>fas</i>	CTAACAACTCTGCCAGCG	GACGGTGGCAGAATTGGATC	MF566098
<i>elovl6</i>	ACATCACCGTGCTGCTACTCCTG	CCGCCACCTGGTCCTTGAGCA	KF857297
<i>ef1α</i>	CTGGAGGGCAGTGAAAAGAT	CATCAAGAGCCTCCAGCAGT	AJ866727
<i>actin</i>	GTGCGTGACATCAAGGAGAA	AGACGCACAATCTTGAGAGCAG	AY148350
<i>l13α</i>	GCTTCGAGGAAATCACCAAG	CAACCTTCCATCCCTTGAAC	DT044539

Résumé du Chapitre 2 en Français

Le chapitre 2 visait à évaluer la conséquence d'une carence sévère en DHA sur les performances métaboliques et de nage du bar européen capturé dans la nature. Un des objectifs était également de caractériser la modulation des voies de synthèse de ces individus au niveau moléculaire. Pour ce faire, des individus G1 de la Seine ont été capturés en 2021 et ramenés au laboratoire. Les poissons ont passé 66 jours en quarantaine pour les acclimater à l'alimentation en granulé et récupérer du stress de la capture et du transport. En Février 2022, le conditionnement nutritionnel a démarré. Les poissons ont été divisés en deux groupes, chacun en triplicat. Le premier groupe était le groupe Control, nourri avec un aliment qui contenait des proportions de DHA supérieure aux besoins du bar Européen (5.3% du total des AG). Le deuxième groupe était le groupe Depleted, nourri avec un aliment qui était presque dépourvu de DHA (0.5% du total des AG). Chaque mois, les individus étaient pesés et mesurés afin de suivre leur croissance. Après 5 mois de conditionnement, les individus ont été soumis à un test en tunnel de nage pour mesurer la vitesse de nage critique (U_{crit}). La consommation d'oxygène a également été mesurée afin d'obtenir des taux métaboliques. Malgré la restriction de l'apport en DHA, les tissus des poissons présentaient des niveaux élevés de DHA, ce qui suggère une rétention sélective ou une synthèse interne. Cependant, cela n'a pas compensé les taux de croissance plus faibles observés chez les poissons dépourvus de DHA. Nous avons également observé une augmentation des gènes liés à la biosynthèse des AGPI-LC, ce qui pourrait indiquer une adaptation métabolique coûteuse. Curieusement, ces changements physiologiques ne se sont pas traduits par des différences dans le taux métabolique ou les performances de nage. L'étude a également révélé une variabilité interindividuelle significative dans la croissance, le taux métabolique et les performances de nage, ce qui justifie une étude plus approfondie des mécanismes sous-jacents et des compromis énergétiques.

***Chapter 3 - Effects of severe DHA
dietary depletion on the escape
response of a wild-caught
euryhaline fish***

Foreword to the Chapter 3

In Chapter 2, we had to select the smallest individuals for the swim tunnel test, because the size of the swimming chamber did not allow us to place fish that were too large (max 23-24 cm). These fish were smaller at the start of the experiment and therefore potentially had a lower growth rate phenotype. To clarify the experimental design, here is the chronology of events:

- **21 Feb. 22 - 02 Aug. 22:** Fish conditioning with Depleted and Control Diets 2/
- **02 Aug. 22 - 01 Sep. 22:** Swimming test experiments (Chapter 2) after 162-192 days of conditioning with Diets 2. Selection of the smallest individuals for the test (**Figure 35**). This led to an abrupt change in the average weight of all remaining individuals, as only the largest individuals remained (**Figure 36**).
- **01 Sep. 22:** Biometry on individuals remaining after the swim test (**Figure 37**). Control n= 31; Depleted n= 26.
- **01 Sep. 22 - 20 Sep. 22:** Conditioning of remaining individuals with Diets 2.
- **20 Sep. 22 - 20 Oct. 22:** Change of diet due to manufacturing concerns (see Material and Methods). Remaining fish (n=62) were then conditioned with Control and Depleted Diet3.
- **20 Oct. 22 - 04 Nov. 22:** Escape response experiments on fish fed the Diets 3 during 30-45 days (Chapter 3). 40 individuals were tested (20 Control and 20 Depleted). Of these 40, we managed to effectively apply the stimulus and obtained a video for 21 (12 Control and 9 Depleted). Growth parameters of the different groups are presented in **Figure 38** and **Table 16**.

- **04 Nov. 22 – 10 Nov. 22** : Sacrifice of the remaining individuals after the escape response experiments (Control = 11, Depleted = 6)

The data from chapter 2 showed some interesting data regarding the amount of DHA in the muscle and the growth rate in the Depleted groups. High DHA level in the muscle was related to a lower growth rate (**Figure 39**). This paradoxical relationship may be explained by the energetic cost of retaining or synthesizing DHA. Given the very strong relationship between these two parameters ($R=0.91$, **Figure 39**), we hypothesised that the growth rate would allow us to estimate the DHA content in the muscle of the Depleted individuals from Chapter 3. By extrapolating the equation for the percentage DHA in muscle / TGC relationship, we therefore created a theoretical percentage of DHA by applying the equation to the TGC of the Depleted individuals from Chapter 3 (**Figure 40**).

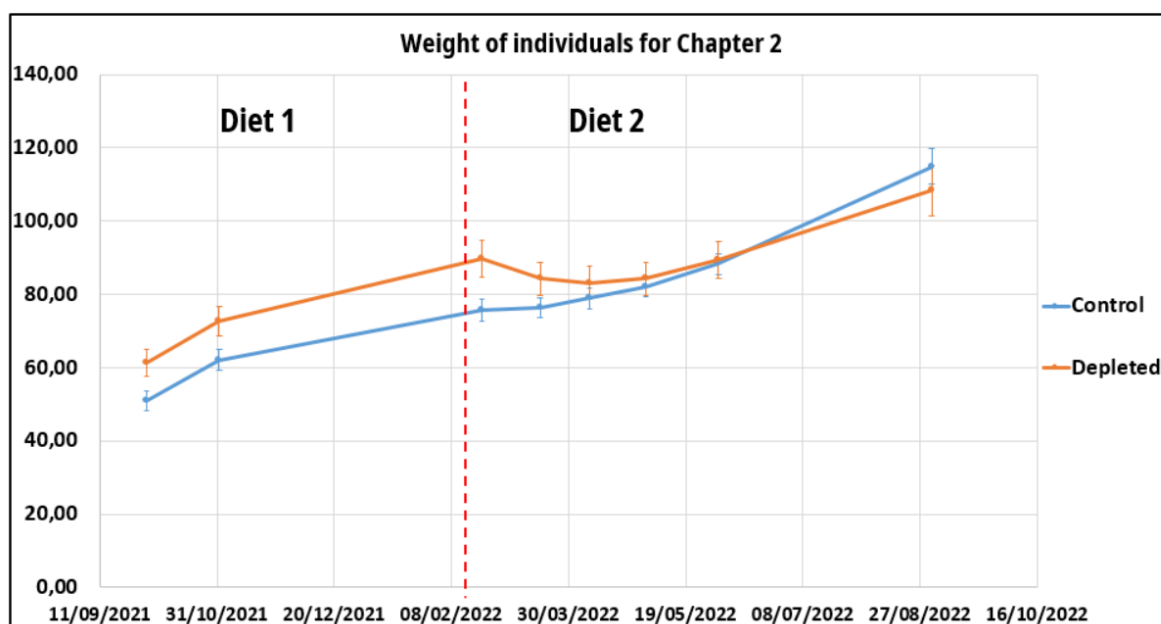


Figure 35: Growth curve of individuals used in Chapter 2, and fed the Diets2. The red dotted line bar represents the feed change from Diets1 to Diets2, and the T0 of the conditioning experiment with the Depleted and Control diets 2 (21 Feb. 2022). Control n=15; Depleted n=16.

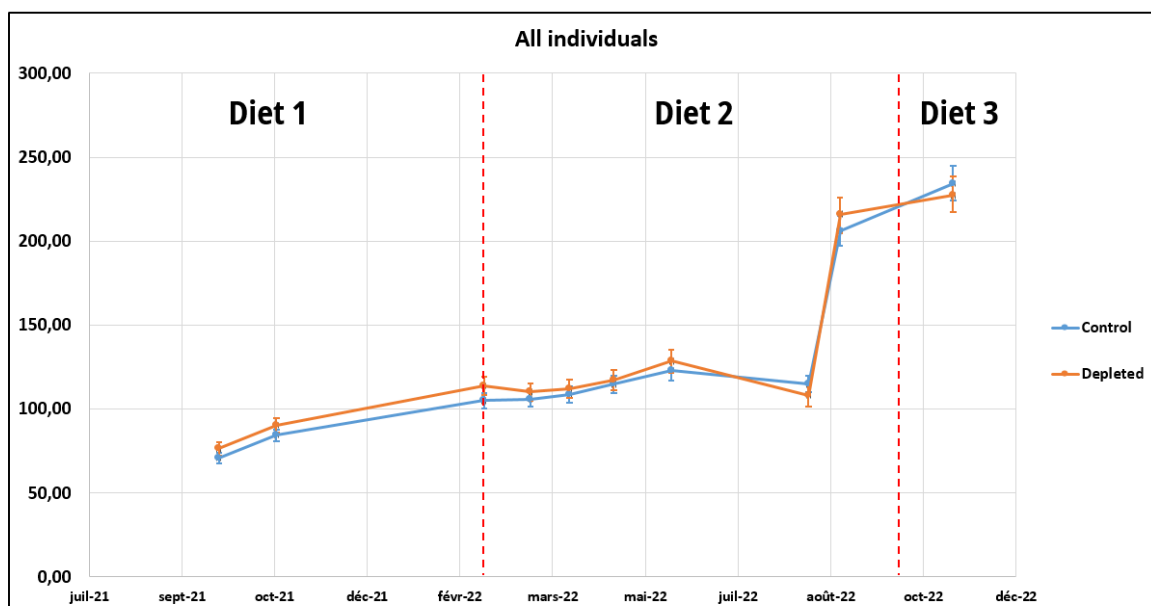


Figure 36: Growth curve for all individuals. The dotted red bars represent the change of feed and the T0 of the conditioning experiment with the Depleted (n=47) and Control (n=47) (21 Feb. 2022). The abrupt change in the curve (01 Sep. 22) corresponds to the end of Chapter 2. After 01 Sep. 22 there were 62 individuals remaining (Control n=32, Depleted n=30).

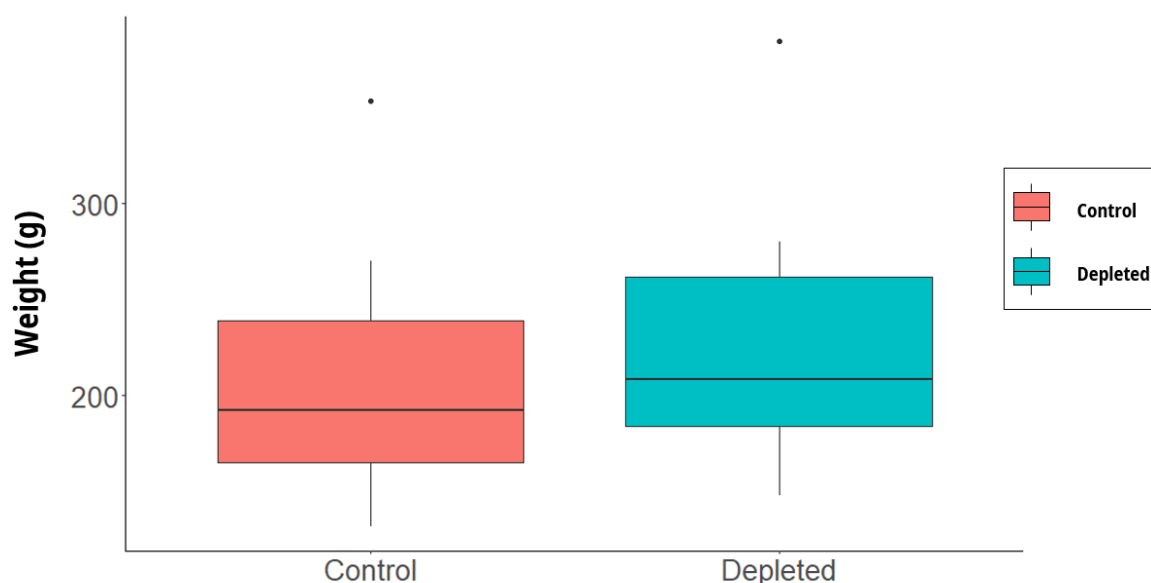


Figure 37: Mean weight of the Control (n=31) and Depleted (n=26) fish during the biometry of 01 Sep. 22 (end of Chapter 2 experiment). The weight was not significantly different between conditions (one-way Anova, p=0.2).

Chapter 3 - Effects of severe DHA dietary depletion on the escape response of a wild-caught euryhaline fish

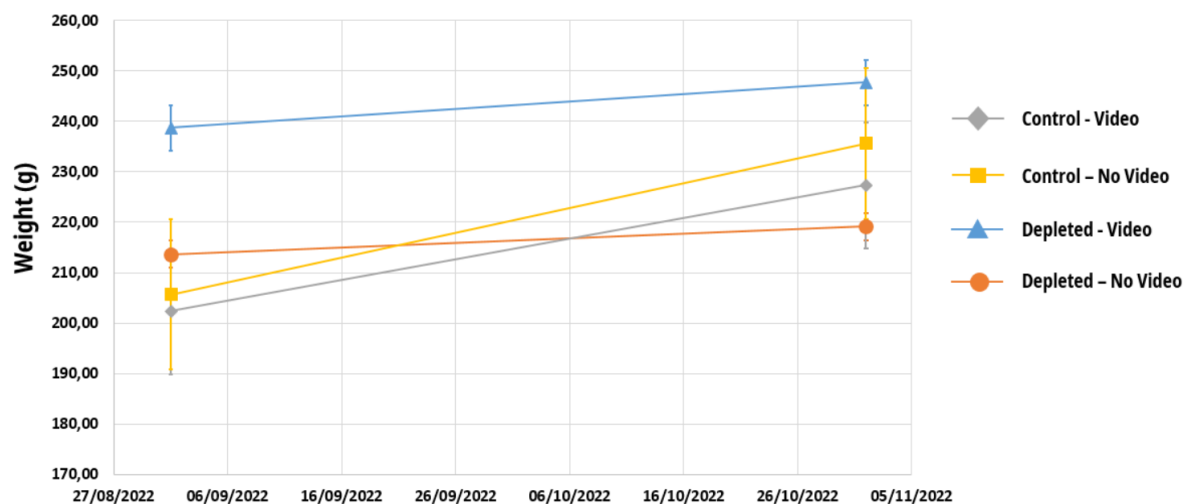


Figure 38: Mean weight (\pm SEM) of the fish from the Escape response experiment (Chapter 3). The first point represents the biometry realized on 01 Sep. 22, and the second dot represents the final weight after the escape response trial (20 Oct 22 - 04 Nov. 22). Yellow lines and squares represent the fish from the Control condition for which no video was obtained (n=8). Blue lines and triangles represent fish from the Depleted condition for which a video was obtained (n=9). Orange lines and circles represent fish from the Depleted condition for which no video was obtained (n=17). Gray lines and diamond represent fish from the Control condition for which a video was obtained (n=19). For all groups, mean fish weight was not different between both dates ($p > 0.05$, one-way Anova).

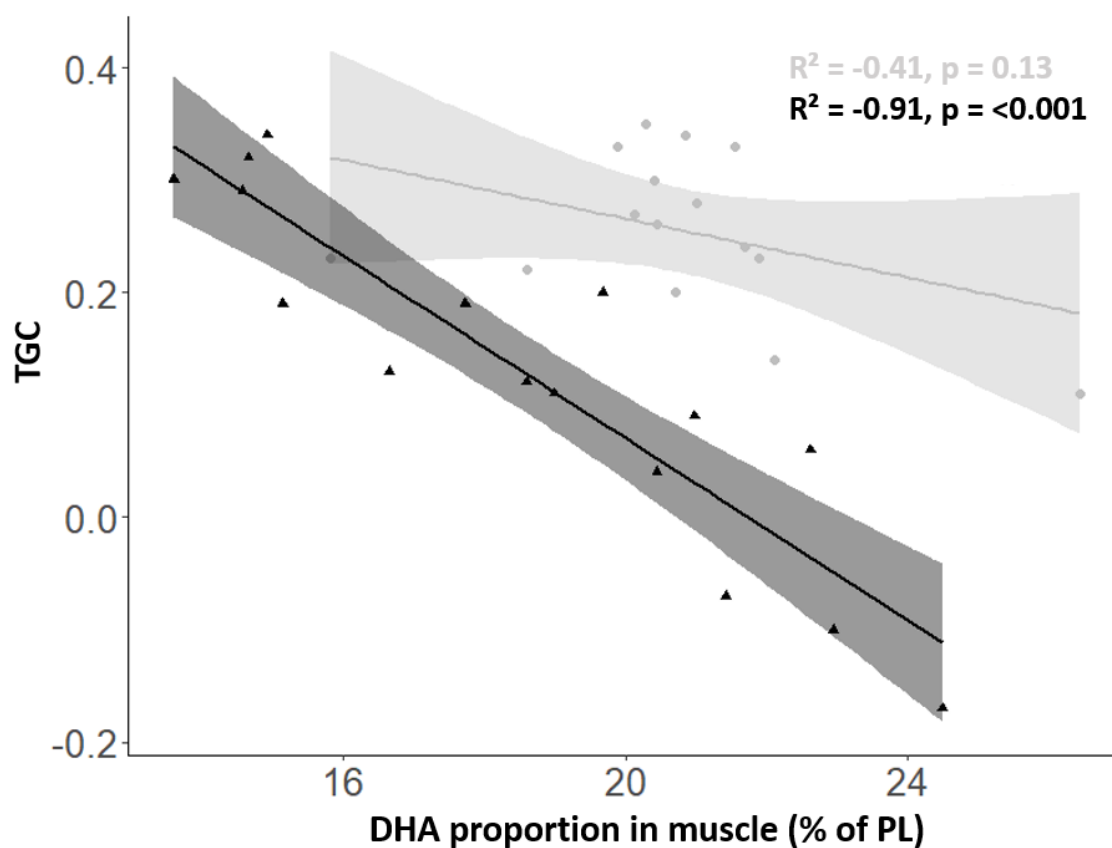


Figure 39: Linear regression between Thermal Growth Coefficient (TGC) and DHA Muscle proportion obtained in chapter 2. The gray regression line represents the Control condition (n=15) and the black regression line represents the Depleted condition (n=16).

Table 16: Mean Weight and TGC of the fish for which no video was obtained. Data are presented as mean \pm SEM. (one-way Anova or Kruskal-Wallis test).

Fish without video	Control (n=19)	Depleted (n=17)	Statistics (p-value)
Weight 01/09	205.7 \pm 12.2	213.7 \pm 10.4	NS
Weight Final	235.6 \pm 14.11	219.1 \pm 13.2	NS
TGC _{Diet2}	0.28 \pm 0.01	0.28 \pm 0.01	NS
TGC _{Diet3}	0.38 \pm 0.05	0.07 \pm 0.06	< 0.001
TGC _{Total}	0.29 \pm 0.01	0.24 \pm 0.01	0.03

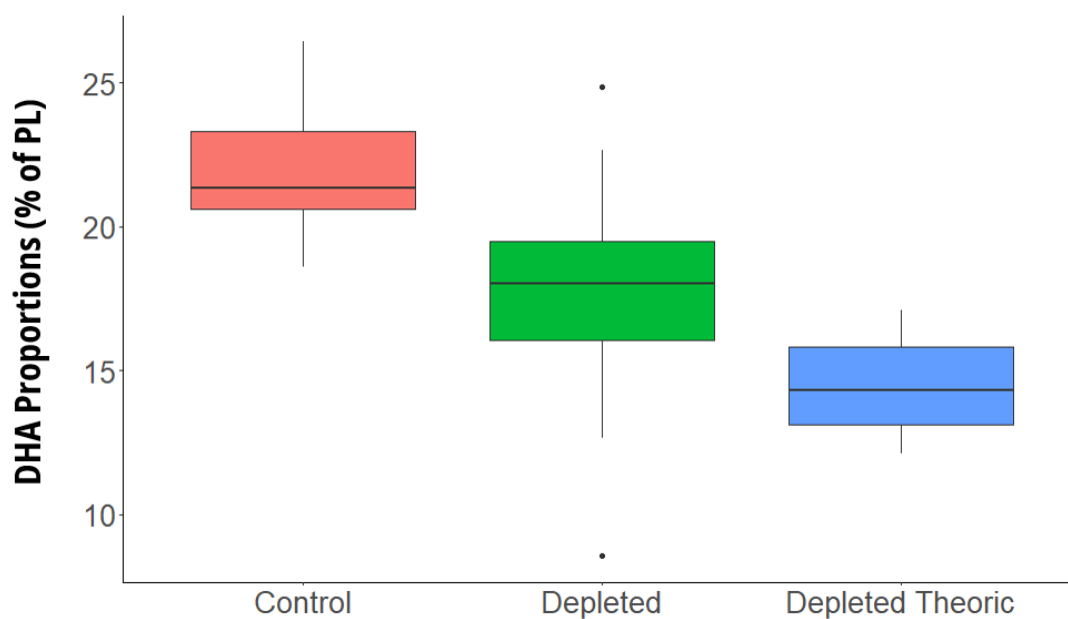


Figure 40: Proportions of DHA in the muscle membrane lipids. “Control” represents the DHA proportion in muscle of fish fed the control diet (Diet 2) in Chapter 2 (n=18). “Depleted” represents the DHA proportion in muscle of fish fed the DHA depleted diet (Diet 2) in Chapter 2 (n=16). “Depleted Theoric” represents the theoretical proportions of DHA obtained from extrapolating the relationship between the DHA proportion in muscle of Depleted fish and the TGC in Chapter 2 and applied to the TGC_{Diet2} of Depleted fish from Chapter3 (n=9).

Research paper n°3 entitled “Effects of severe DHA dietary depletion on the escape response of a wild-caught euryhaline fish”

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1. Abstract

Chapter 3 aimed to investigate the impacts of DHA deficiency on the ability of juvenile wild sea bass to evade predators. For this, specimens were conditioned for 6 months on a diet virtually devoid of DHA (0.5% TFA), while a control group received a diet surpassing the DHA requirements of this species (5.3% DHA). After 5 months, 36 fish (18 from each group) were subjected to a swimming tunnel test (Chapter 2) and subsequently sacrificed. Due to the tunnel size, smaller fish were selected, leading to a sudden increase in the average weight of remaining fish in the tanks. After 6 months on Diet 2 and an unexpected 30-day period on a still-deficient Diet3 with slightly higher DHA, fish underwent a predatory escape response test, measuring variables such as stimulus response, latency time, distance covered in 70 ms, and turning rate. Contrary to the reduced growth rate observed for the Depleted group in Chapter 2, there was no difference during the Diet 2 period, possibly due to the inadvertent selection of smaller, slower-growing individuals. However, a surprising decline in growth occurred with Diet3, even though it had more DHA. Given its rapid onset, this might be attributed to stress factors like altered food texture and reduced tank populations after Chapter 2 sampling. Nevertheless, only the DHA-deprived group exhibited this reduced growth, suggesting a potential link between DHA deficiency and stress response. While no significant effects were observed for behavioral variables, a trend towards decreased responsiveness and increased latency was noted in the DHA-deprived fish. A trend was also identified between brain size and covered distance. These findings hint at a potential DHA deficiency impact on predatory

evasion abilities. Analyzing tissue fatty acid content in correlation with growth and behavioral variables could elucidate the raised concerns.

2. Introduction

The role of omega-3 fatty acids (LC n-3 PUFA) has been universally recognized for being vital for physiological and neurological health in vertebrates (Simopoulos 1991). LC n-3 PUFA are major components of phospholipids (main contributors to polar lipids; PL), thus regulating membrane functions (Sargent et al., 2003). They possess beneficial effects on growth, reproduction, cognitive functions, disease prevention and play a key role in cell signaling in mammals and fish (Sargent et al., 2003; Tocher, 2003). The dietary FA composition has been shown to modulate several behavioral parameters in mammals such as stress vulnerability, latency or exploring new environments (Fedorova and Salem 2006). Among LC n-3 PUFA, docosahexaenoic acid (DHA) is considered as the most important fatty acid (FA) for visual and cognitive functions in vertebrates (Masuda et al. 1999; Bazinet and Layé 2014). It is a key component of PL in the membranes of the brain and retina of vertebrate, including fish (Mourente et al. 1991; Lauritzen et al. 2001).

Despite their biological importance, endogenous synthesis of LC n-3 PUFA is rather limited in marine fish due a low activity of desaturase and elongase, enzymes necessary to the biosynthesis process from endogenous precursors (Turchini et al. 2009; Tocher 2015). Fish must then obtain them through their diet. In the natural environment, LC n-3 PUFA are produced at the basis of the aquatic food webs by micro algae and subsequently transferred to higher trophic levels including fish (Arts et al. 2001). Yet global change encompassing factors such as warming of sea surface temperature or ocean acidification is predicted to reduce LC n-3 PUFA and particularly DHA production by primary producers, (Galloway and Winder, 2015; Hixson and Arts, 2016). Thus, a limitation in dietary DHA could have cascading effects on fish neural development and behavior. Indeed, a non-optimal composition of neuron cell membranes may affect the brain function resulting in the alteration of behavior in fish fed low levels of DHA (Benítez-Santana et al. 2014). However, there are few studies that investigated the link between limitation of LC n-3 PUFA and behavioral responses in fish (Benítez-Santana et al. 2014; Lund et al. 2014; Carvalho et al. 2022).

Behavior is dictated by the relationship between the environment and the nervous system that will induce a response/reaction to a specific event (i.e stimulation) (Lund et al. 2014). In the wild, impairment of behavioral parameters, such as the ability to escape from predators, could directly influence fish survival. Indeed, escape response determines the predator-prey relationships and allows the fish to run away from a threat thus increasing their chance of survival (Domenici and Blake 1997). Escape response in fish consists in a rapid movement called “C-start” initiated by Mauthner cells located in the hindbrain (Domenici and Blake 1997; Korn and Faber 2005). The escape response depends on a number of variables such as latency, acceleration, turning rate or distance that, in the end, dictates how a fish escapes from a predator (Domenici et al. 2007). All these variables are activated through neural activity, highlighting the importance of a normal neural activity to allow fish to avoid predators (Domenici and Hale 2019). Recent studies have found that a diet low in LC n-3 PUFA negatively impacts the escape behavior and nerve cell responses in gilthead seabream larvae (*Sparus aurata*) (Benítez-Santana et al. 2012). Specifically, a deficiency in docosahexaenoic acid (DHA) impairs the larvae ability to react to sound (Benítez-Santana et al. 2012, 2014). The negative effect of LC n-3 PUFA limitation was also noticed in juvenile pikeperch (*Sander lucioperca*) after both 1 month and 3.5 months dietary conditioning (Lund et al. 2014). Only information is available on the effects of a dietary LC n-3 PUFA limitation in the escape response of juvenile marine fish (Carvalho et al. 2022). A reduced dietary LC n-3 PUFA (3.35% EPA+DHA) induced an upregulated neurogenesis and neural activity, but did not impact the locomotor behavioral response of *S. aurata* juveniles (Carvalho et al. 2022).

Because fish cognition and behavior is a relatively recent field of studies, most studies have been conducted on farmed or domesticated fish (Salena et al. 2021), which can have different behavior or performance compared to their wild counterparts (Beamish 1978; Walker et al. 2005). Using wild fish as a model for behavioral studies could be a way to gain further knowledge on fish behavior with ecological relevance (Salena et al. 2021; Bshary and Triki 2022).

In this context, the objective of the present study was to assess the effects of LC n-3 PUFA dietary deficiency on the escape behavior of wild-caught European sea bass (*Dicentrarchus labrax*), a euryhaline species of ecological and economic interest. We chose to focus on the

DHA deficiency in the diet, as it is a major component of sea bass in liver, muscle and brain (Chap 1) and because only few investigations were carried out using DHA depletion only (uncoupled with EPA). European sea bass has already been shown to modulate its LC n-3 PUFA synthesis capacity at the juvenile stage when subjected to a deficient diet at the larval stage (Vagner et al. 2007b). In addition, the European sea bass is a species, which, at the juvenile stage, has a gregarious behavior and is subject to predation pressure by bigger fish (Pickett and Pawson 1994).

Using long-term nutritional conditioning of wild-caught sea bass, we experimentally investigated the effects of severe dietary DHA limitation on the escape response to a mechano-sensory stimulus.

3. *Material and Methods*

3.1. Ethical statement

All of the experiments were performed with respect to the regulations of the Animal Care Committee of France (ACCF). They 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.

3.2. Animals

3.2.1. Collection

Juvenile European sea bass (first year) were captured using a trawl in the Seine estuary during the NOURDEM (Le Goff et al. 2022) survey in August 2021. Around 150 fish were then transported to Ifremer rearing facilities (Plouzané, France) into an oxygenated 400 L tank containing the water of the environment in which they were caught.

3.2.2. Rearing and experimental diets

Fish were acclimated 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. Fish 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 these 30 days, 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) in open circuit, exposed to a 12h light / 12h dark photoperiod cycle, and natural temperature. Before being transported to their tank, fish were anesthetized using MS-222 (tricaine methanesulfonate, $0.1 \text{ g}\cdot\text{L}^{-1}$), individually weighed, measured and pit-tagged (M120; biolog-id, Réseautomatique, Bernay, France). Fish were then progressively (from commercial pellets to experimental diet in 1 week) fed with two experimental iso-proteic and iso-lipidic diets (**Table 17**) differing by their FA contents (Diet 2; **Table 18**, $\text{DHA}_{\text{Depleted}}$: 0.5% Total FA (TFA); $\text{DHA}_{\text{Control}}$: 5.3% TFA). Both diets were manufactured at INRAE (Donzacq, France). A control and a low DHA diets were formulated using fish oil and microalgae oil (*Nannochloropsis spp.*), respectively. Fish were fed from 21 of February 2022 to 20 of September 2022 with these diets (*i.e* 211 days). Fish were then fed with another diet (Diet 3; **Table 18**, $\text{DHA}_{\text{Depleted}}$: 2.5% TFA; $\text{DHA}_{\text{Control}}$: 7.6% TFA) from 20 of September to the end of the experiment (*i.e* 45 days) because of a manufacturer issue. The two conditions (later called Control and Depleted, respectively) were reared in triplicates ($n= 51$ fish for control condition and 50 for depleted condition). Fish were fed the diet once a day (0.6-2% of total tank biomass, depending on water temperature that followed the natural temperature of the bay of Brest: $14.5^\circ\text{C} \pm 0.14$, min-max = 10.3°C -

18.7°C) for a minimum of seven months. Oxygen saturation in the tanks was daily controlled and was never lower than 90%.

Table 17: Formulation of the two experimental diets (Control and Depleted). Formulation for Diet 2 and Diet 3 was the same. The components had different sources.

Ingredients (g.100g ⁻¹)	Control Diet	Depleted Diet
Fish Meal ¹	20	20
Wheat gluten ²	50	50
Canola oil ³	3	5
Fish oil ⁴	7	0
<i>Nannochloropsis spp.</i> Oil ⁵	0	5
Starch ²	6	6
Vitamins ⁶	4	4
Minerals ⁷	2	2
Cellulose	5	5
Betaine	3	3
Chemical composition (g.100g ⁻¹ ± SD) - Analysed by UpScience		
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

¹ Diet 2: LT70 Sopropêche, Diet 3 : LT70 Sopropêche. ² Diet 2 : Roquette, Lestrem

³ Diet 2: Oléandes, Mugron, France; Diet 3: Oléandes, Mugron, France

⁴ Diet 2 : Polaris, Quimper, France ; Diet 3 : 70Durable, Sopropêche

⁵ Diet 2 : Polaris, Quimper, France ; Diet 3 : Polaris, Quimper, France

^{6,7} See Appendix

Chapter 3 - Effects of severe DHA dietary depletion on the escape response of a wild-caught euryhaline fish

Table 18: Fatty acids composition of the experimental diets given from 21 Feb. 2022 to 20 Sep. 2022 (Diet 2) and from 20 Sep. 2022 to the end of the experiment (Diet 3). Data are presented as mean \pm sd (n=3). 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.

FA	Control Diet 2	Control Diet 3	Depleted Diet 2	Depleted Diet3
14:0	4.9 \pm 0.5	2.7 \pm 0.0	2.2 \pm 0.5	2.0 \pm 0.0
15:0	0.3 \pm 0.5	0.3 \pm 0.0	0.2 \pm 0.5	0.2 \pm 0.0
16:0	15.1 \pm 1.2	15.4 \pm 0.1	12.8 \pm 1.0	13.6 \pm 0.2
17:0	0.1 \pm 0.5	0.3 \pm 0.0	0.1 \pm 0.5	0.2 \pm 0.0
18:0	3.2 \pm 0.5	2.8 \pm 0.0	1.2 \pm 0.5	1.3 \pm 0.0
20:0	0.4 \pm 0.5	0.4 \pm 0.0	0.4 \pm 0.5	0.3 \pm 0.0
22:0	0.2 \pm 0.5	0.2 \pm 0.0	0.2 \pm 0.5	0.2 \pm 0.0
Σ SFA	24.8 \pm 1.0	21.9 \pm 0.1	17.9 \pm 1.5	17.8 \pm 0.2
16:1n-7	4.8 \pm 0.5	4.1 \pm 0.0	9.4 \pm 0.8	8.5 \pm 0.1
16:1n-9	0.1 \pm 0.5	0.3 \pm 0.0	0.5 \pm 0.5	0.6 \pm 0.0
18:1n-7	2.8 \pm 0.5	2.8 \pm 0.0	2.0 \pm 0.5	2.0 \pm 0.0
18:1n-9	22.7 \pm 1.8	24.7 \pm 0.2	30.0 \pm 2.4	26.5 \pm 0.3
20:1n-9	1.5 \pm 0.5	1.2 \pm 0.0	0.6 \pm 0.5	0.7 \pm 0.0
Σ MUFA	35.7 \pm 2.3	35.0 \pm 0.1	43.8 \pm 2.6	39.1 \pm 0.3
18:2n-6	12.6 \pm 1.0	17.9 \pm 0.2	17.0 \pm 1.4	18.6 \pm 0.3
18:3n-3	2.9 \pm 0.5	3.9 \pm 0.0	5.1 \pm 0.5	4.5 \pm 0.0
20:4n-6	0.8 \pm 0.5	0.7 \pm 0.0	1.5 \pm 0.5	1.5 \pm 0.0
20:5n-3	10.7 \pm 0.9	6.5 \pm 0.0	13.2 \pm 1.1	12.2 \pm 0.1
22:5n-3	1.5 \pm 0.5	1.0 \pm 0.1	1.2 \pm 0.5	0.2 \pm 0.0
22:6n-3	5.3 \pm 0.5	7.6 \pm 0.1	0.5 \pm 0.5	2.5 \pm 0.0
Σ PUFA	39.6 \pm 2.5	42.5 \pm 0.2	39.5 \pm 2.4	42.0 \pm 0.4
Σ LC PUFA	18.6 \pm 1.2	21.5 \pm 0.2	15.7 \pm 1.0	16.6 \pm 0.1
Σ n-3	22.7 \pm 0.5	19.4 \pm 0.2	19.9 \pm 1.0	20.2 \pm 0.1
Σ n-6	14.0 \pm 1.1	19.4 \pm 0.2	19.3 \pm 1.2	20.8 \pm 0.2
EPA/DHA	2.0 \pm 0.1	0.9 \pm 0.0	26.4 \pm 0.5	4.8 \pm 0.2
ARA/EPA	0.07 \pm 0.0	0.11 \pm 0.0	0.11 \pm 0.0	0.12 \pm 0.0

3.3. Growth performance

Every month, 24h-starved fish were anesthetized with MS-222 (0.1 g.L⁻¹) and then weighed (\pm 0.1 g) and measured (\pm 0.1 cm).

For each individual, the Thermal Growth Coefficient (TGC; g.degree day⁻¹) was calculated using the following equation:

$$TGC = 1000 \times \left(\frac{W_{final}^{1/3} - W_{initial}^{1/3}}{T \times D} \right)$$

Where W_{final} is the weight at the end of the experiment, $W_{initial}$ is the weight at the beginning of the experiment, T is the average temperature during the experiment (Diet 2 = 15.5°C; Diet 3 = 16.6-16.0°C) and D is the number of days between the disposal into experimental condition and the end of the experiment (Diet 2: 192days; Diet 3: 30-45 days).

The TGC_{Diet2} was calculated from 21 Feb. 22 to 01 Sep. 22. The TGC_{Diet3} was calculated from 01 Sep. 22 to the day of the test. The TGC_{Total} was calculated from 21 Feb. 22 to the day of the test.

Fulton condition factor (CF, arbitrary unit) was also calculated for Diet 2 (Weight and TL at 01 Sep. 22) and Diet 3 (Final weight and Total length) of conditioning using the following equation:

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

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

3.4. Escape response experiment

After long-term nutritional conditioning (from 241 to 256 days), 20 fish per treatment (Depleted: 244.1 \pm 24.9 g, 27.3 \pm 1.0 cm; Control: 230.1 \pm 16.3 g, 26.4 \pm 0.6 cm) were randomly selected to be tested for an escape response experiment. Prior to the test, fish were isolated and fasted during a minimum of 48 hours. Each day, two fish from both dietary conditions were tested in the morning (from 9am to 1pm) to avoid any differences linked to chronobiology. Fish were anesthetized in a bucket (MS-222; 0.1 g.L⁻¹) transferred from their isolation tank to the experimental tank and were left acclimated for one hour to reduce stress

and allow them to explore their new environment. The experimental tank was a white-bottom tank, similar to that in which they were reared during 5 months, which was covered by a wooden plank and in which LED lights were placed. After the acclimation period, the fish were submitted to a mechano sensory stimulus that consisted in a Falcon tube filled with sand (43 g) that was attached to an electro-magnet connected to a switch. Turning the switch off allowed the drop of the stimulus, attached to a string, to drop through a PVC tube inside the water without touching the bottom of the tank. This tube hides the stimulus so the fish did not notice it until it touched the water, imitating an avian predator in a turbid environment (Lefrançois et al. 2005). This allowed us to observe the exact moment of stimulation (i.e the video frame when the stimulus touched the water). Simultaneously to the triggering of the switch, a video recording the escape response was manually started using a high speed camera (AOS S-MOTION, 500 frames per second, AOS Technologies, Switzerland), and using the OAS Imaging Studio software. To monitor the fish behavior before triggering the switch, a webcam (Logitech C920s PRO) was placed inside the tank and connected to a laptop.

The triggering of the switch required that:

- The fish was positioned within the camera field of view not too close to the stimulus
- The speed of the fish must be null or very slow
- The surface water must be still and without waves

If after 13:00, these conditions were not fulfilled, the fish was taken out of the experimental tank without being tested again. We then managed to activate the stimulus and record escape response for 12 fish and 9 fish from the Control and the Depleted groups, respectively.

3.5. Video analysis and calculation of parameters

All of the videos were analyzed with the software Kinovea (V0.9.5). The video analysis allowed us to measure several escape response variables:

- Responsiveness

The responsiveness represents the proportion of the fish that escape from the predator on the total fish that have been stimulated (Domenici and Hale 2019).

- Latency

The latency corresponds to the time (in ms) between the moment when the stimulus is visible and the moment when the fish triggers an escape response (*i.e* the frame when the first movement of the fish is visible).

- Distance covered

The distance covered (in cm) corresponds to the distance between the center of mass (CM) of the fish at the frame before the escape response was triggered and the CM of the fish 70 ms later (Domenici et al. 2007). The CM of the fish was determined to be at 38% of the length of the fish, starting from the tip of the head (Lefrançois and Domenici 2006).

- Duration of stage 1

Stage 1 (S1) represents the first part of the escape response (C-Start), sometimes followed by a second bend (Stage 2). Stage 1 is considered to be over at the moment when a change of direction occurs (Domenici et al. 2007).

- Turning rate

Turning rate (in degrees s^{-1}) represents the angular velocity of the escape response (Domenici and Hale 2019). In other words, it is how fast a fish turns after triggering the escape response. It is the ratio between the turning angle of stage 1 and the duration of stage 1 (Domenici et al. 2007).

3.6. Sample collection

After the escape response test, fish were caught with a net and transferred in a bucket of seawater and euthanized (MS-222, 0.4 g.L⁻¹). Then, the fish were individually weighed and measured. The muscle (on the left side, just behind the head), the liver (without gallbladder), the brain and the eyes were sampled for further fatty acid. The brain and the liver were weighed to calculate the cerebroomatic index (CSI, %) and hepatosomatic index (HSI, %) using the following equations :

$$CSI = \left(\frac{W_{brain}}{W_{fish}} \right) \times 100$$

$$HSI = \left(\frac{W_{liver}}{W_{fish}} \right) \times 100$$

3.7. Statistical analysis

Data analysis was conducted on RStudio (V4.2.1). Growth parameters (TGC, Fulton's K, HSI and CSI) and escape response variables (except Responsiveness) were tested using one-way ANOVA with Condition as the explanatory variable with two levels (Control or Depleted). ANOVA assumptions were tested using Shapiro-Wilk and Bartlett tests to test for normality and homogeneity, respectively. If the ANOVA assumptions were not met, a Kruskal-Wallis test was used instead. No data transformation was applied. Responsiveness was tested with a Fisher exact test. Correlation between CSI and Distance covered was tested with a Pearson correlation.

4. Results

The analysis of the FA composition was not completed before the finalization of this manuscript. Therefore, only the data on growth and escape response will be discussed.

4.1. Growth performance

Table 19 presents the data on growth performance of the 9 Depleted fish and the 12 Control fish. Over the 211-day period during which these fish were fed the Diet 2, thermal growth coefficient (TGC) was not different between the Control and Depleted groups. TGC_{Total} also did not differ between the two groups. However, during the 30-45 day period during which fish were fed Diets 3, the TGC was notably lower for fish on the Depleted diet.

The CF for the duration of diet 2 was not significantly different between both dietary groups. However, a non-significant trend ($p=0.09$) was observed for the CF measured at the end of the experiment (**Table 19**).

Neither initial nor final weights or heights were different between the two fish groups (**Table 19**). The growth curve for the duration of the experiment is presented in **Figure 41**.

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Table 19: Growth performance, Fulton's K, cephalosomatic (CSI) and hepatosomatic (HSI) indice for control (n=12) and depleted (n=9) conditions. Data are presented as mean \pm SEM.

	Control	Depleted	p-value
TGC _{Diet2}	0.31 \pm 0.03	0.28 \pm 0.03	0.89
TGC _{Diet3}	0.31 \pm 0.07	0.12 \pm 0.05	0.004
TGC _{Total}	0.29 \pm 0.01	0.28 \pm 0.03	0.24
CF Diet 2	1.25 \pm 0.01	1.25 \pm 0.01	0.84
CF Final	1.22 \pm 0.02	1.17 \pm 0.02	0.09
Initial weight	117.5 \pm 5.3	142.2 \pm 12.7	0.18
Final weight	230.1 \pm 16.3	244.1 \pm 24.9	0.63
Initial TL	21.5 \pm 0.3	22.6 \pm 0.7	0.14
Final TL	26.4 \pm 0.6	27.3 \pm 1	0.42
HSI	2.1 \pm 0.1	1.7 \pm 0.2	0.08
CSI	0.15 \pm 0.01	0.15 \pm 0.01	0.71

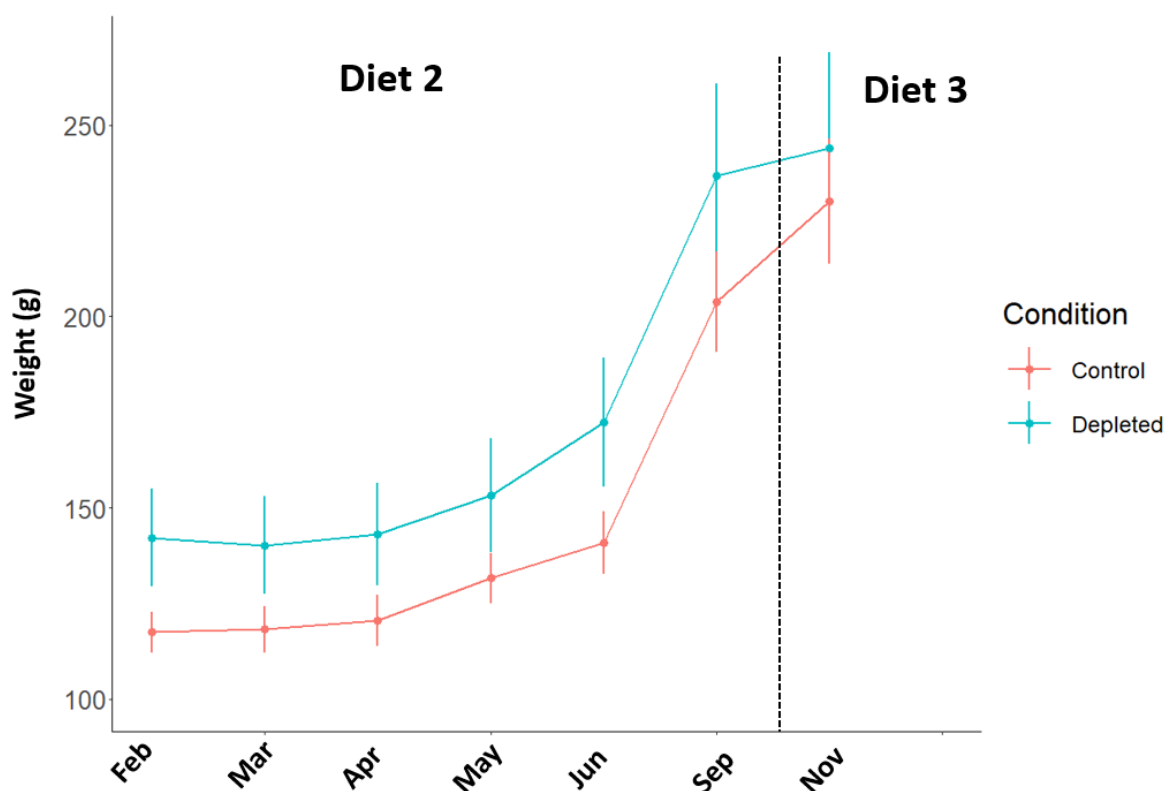


Figure 41: Growth curve representing weight (g) over the duration of the experiment Control: n= 12, Depleted n=9. The black dotted line represents the change from Diet 2 to Diet 3.

4.2. Escape response parameters

No significant results were observed for the measured behavioral variables (Responsiveness, Latency, Distance, Duration of S1 and Turning rate) (**Figure 42**). However, a non-significant trend was observed for responsiveness (Control = 91.7%; Depleted = 55.6 %; $p=0.12$, Fisher test) and latency (Control = 52.73 ± 8.1 ms; Depleted = 71.20 ± 13.1 ms; $p=0.19$, Kruskal-Wallis test) (**Figure 42**). When considering all individuals, the CSI showed a non-significant trend with the distance covered ($p = 0.08$; **Figure 43**; Pearson correlation).

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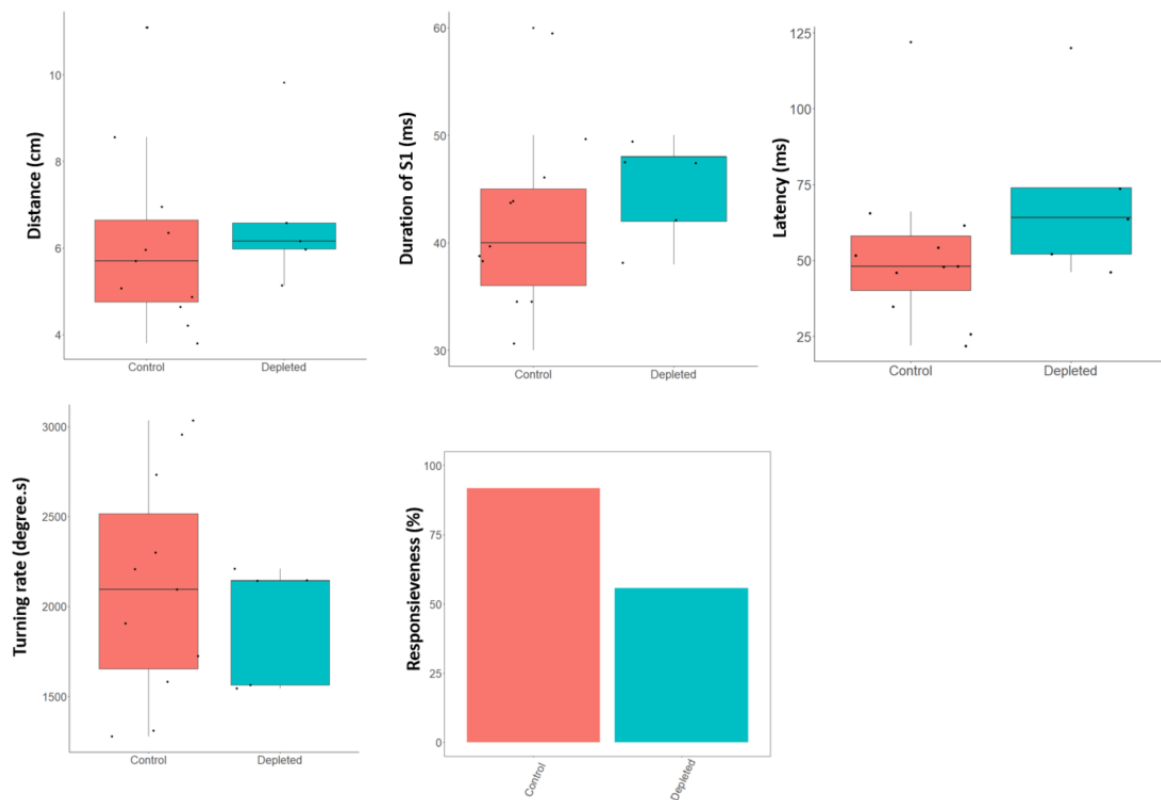


Figure 42: Behavioral parameters measured in both experimental conditions. The blue bars represent the control condition (n=12), and the red bars represent the depleted condition (n=9). Data are presented as mean ± SEM.

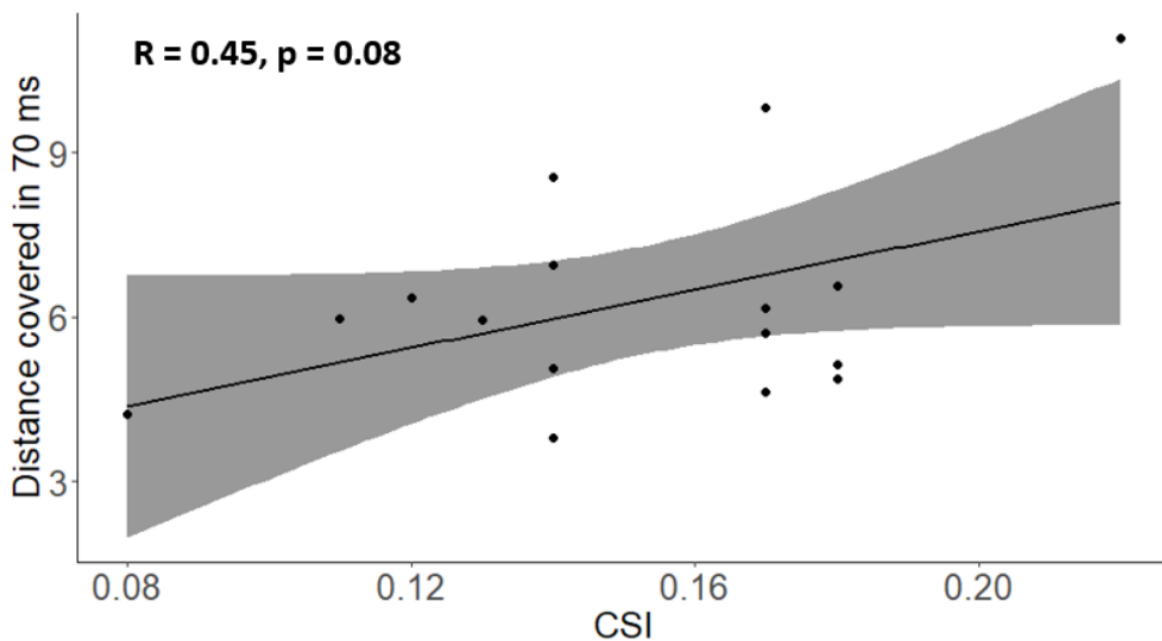


Figure 43: Pearson's correlation between the distance covered by the fish in 70 ms (cm) and the cerebrosomatic index (CSI; %) for depleted fish (n=5) and control fish (n=11).

5. Discussion

This study is one of the first investigating the effects of DHA dietary limitation on the escape response of juvenile fish. To our knowledge, this is also the first time that it was done on wild-caught fish.

In chapter 2, we showed a deleterious effect of 5 months conditioning on the Depleted diet on growth. In the present study (chapter 3), this negative effect on growth disappeared despite the fact that the individuals were fed the same diets (Diet 2) for 6 months (21 Feb. 2022 - 01 Sep. 22). This difference is probably due to the experimental design used for Chapter 3. Indeed, as explained in the preamble to this chapter, the smallest individuals, and therefore potentially those with the lowest growth rate, were chosen for Chapter 2. This therefore induced a bias, which may explain the lack of difference between the two conditions, despite the fact that the individuals were fed the same food for one month longer than in Chapter 2.

However, the reduction in TGC when the Depleted individuals were fed Diet 3 was surprising. In fact, this diet contained five times more DHA than Diet 2 but resulted in a reduced growth compared with the control group. This reduction in TGC occurred rapidly (30-45 days), suggesting a behavioral effect rather than a nutritional effect. Indeed, if after 6 months, the deficient feed did not reduce growth, it is unlikely that a feed containing more DHA would have done so in 30 days. The texture and palatability of Diet 3 was probably different from that of Diet 2 because it was not manufactured in the same way. However, the two Diet 3 (Control and Depleted) had a similar texture, but only the Depleted individuals showed a reduced growth. This could be explained by a greater vulnerability to change in DHA-deficient individuals, for which the stressful effect of the change in diet coupled with the reduction in the number of individuals per tank (because of those used for Chapter 2). In addition, an inter-individual variability of the TGC of the Depleted fish fed with Diet 3 was much higher compared to that of fish fed the Diet 2 (CV= 209% vs 30%, data not shown). These results were also visible for the Control condition but to a lesser extent (CV = 50% vs 34%). It is important to note that the conditioning with Diet 2 (192 days) was much shorter than the one with Diet 3 (130-45 days) which could be an explanation for this high inter-individual variability as the fish might not have had time to acclimate to this new diet.

The dietary conditioning did not induce differences in the efficiency to escape from a predator. However, we found a non-significant trend of reduced responsiveness in fish fed the Depleted diet. Considering that only five out of the nine individuals from the Depleted conditions reacted to the stimulus, this led to a reduced statistical power to test the other variables. This trend was also present for latency in the Depleted group, suggesting a longer reaction time, which could be due to an increase in the duration of the nerve signal to activate escape. A high degree of inter-individual variability was also observed, as it is often the case in behavioral studies (Benhaïm et al. 2012, 2013b, a). This inter-individual variability could be exacerbated by the use of wild fish, which have a different genotype and phenotype to domesticated fish (Milla et al. 2021). The use of wild individuals is recommended in cognition experiments because it increases the relevance of the study, due to the effects of behavioral modifications linked to domestication (Milla et al. 2021; Salena et al. 2021; Bshary and Triki 2022). Considering the small number of individuals that positively responded to the stimulation (Depleted = 5, Control = 11), this inter-individual variability could smooth the differences between groups.

Feeding on a low DHA diet did not impair the liver and brain size. Although, a non-significant trend was observed for HSI indicating a possible effect of DHA depletion. HSI can be an indicator of the welfare of fish and nutritional status (Sheridan and Kao 1998; Brown and Murphy 2004). In addition, a strong non-significant trend was observed between HSI and CF, a proxy for fish well-being (Mozsár et al. 2015). HSI is often correlated with lipid levels, particularly reserve lipids (Sheridan 1988). Therefore, a higher HSI in Control fish could mean a better capacity to store reserves. DHA is involved in the development of the brain and is usually associated with a higher brain size (Ishizaki et al. 2001; Závorka et al. 2022). DHA is also the most important FA for cognition and behavior in both mammals and fish (Innis 2007; Pilecky et al. 2021). Although not significant ($p=0.08$), an interesting positive trend between CSI and distance covered by the fish was found. This result implies that while CSI was similar between groups, fish with smaller brains would flee on a shorter distance than fish with bigger brains. A shorter distance covered could have a detrimental effect on the survival of fish in the wild (van der Bijl et al. 2015). The similarity in brain size between the two groups could be explained by the fact that the brains of these fish are already fully developed. In juvenile fish,

the brain is already fully developed, making it less sensitive to nutritional variations than larvae (Carvalho et al. 2022).

6. Conclusion

In conclusion, we observed paradoxical results of DHA depletion on the fish growth that might have been influenced by the experimental design. Though the effect of the diet was not significant, we highlighted trends that could impaired the ability to escape a predator in the Depleted group. We observed a non-significant relationship between the measured variables and organ size. This opens the question on the link between the growth and behavioral variables with the FA composition of the tissues. In addition, further studies should focus on using ecologically relevant models such as wild-caught fish to improve knowledge about the stress response of fish in its environment.

Résumé du Chapitre 3 en Français

Le Chapitre 3 avait pour but de tester les effets d'une carence en DHA sur la capacité de bars juvéniles sauvages à échapper à un prédateur. Pour ce faire nous avons conditionné les individus pendant 6 mois avec une diète presque dépourvue de DHA (0.5% TFA). Un groupe contrôlé a été nourris pendant la même durée avec un aliment au-dessus des besoins en DHA de cette espèce (5.3% de DHA). Après 5 mois, 36 individus (18 de chaque condition) ont été soumis à un test en tunnel de nage (Chapitre 2) et sacrifiés. Étant donné la taille des tunnels, les plus petits individus ont dû être sélectionnés pour ce test. Cela a créé une augmentation brutale dans le poids moyen des poissons restant dans les bacs. Le conditionnement nutritionnel s'est poursuivi de la même manière qu'avant le test de nage. Au bout de 6 mois, suite à un problème d'aliment, les poissons ont été nourris pendant 30j avec un aliment contenant plus de DHA mais toujours carencé (chiffres). Tous les mois, les poissons étaient pesés et mesurés afin de suivre leur croissance. Après 6 mois de Diet2 et 30j de Diet3, les poissons ont été soumis à un test de réponse de fuite. Ce test consistait à tester plusieurs variables liées à la capacité à échapper à un prédateur. Ces variables étaient: la réponse ou non au stimulus, le temps de latence dans la réponse au stimulus, la distance parcourue en 70 ms et le turning-rate. Le taux de croissance plus faible observé chez les Depleted dans la Chapitre 2 ne s'est pas retrouvé si l'on considère la période Diet2. Ce résultat peut s'expliquer par la sélection indirecte des individus les plus petits et donc potentiellement avec la croissance la plus faible. Cependant, une diminution du taux de croissance a été observé avec la Diet 3. Cette diminution n'était pas attendue car la diète contenait plus de DHA. Vu la rapidité de la diminution de croissance (30j), il est probable que cette diminution de croissance soit due à un stress. La texture différente de l'aliment ainsi que la diminution des individus dans les bacs dus aux prélèvements du chapitre 2 sont des éléments stressant. Cependant, ces paramètres étaient présents pour les deux groupes et les individus control n'ont pas eu de croissance réduite. Ainsi, il existe peut-être un lien entre carence en DHA et réponse au stress. Aucun effet significatif n'a été observé pour les variables comportementales. Cependant une tendance de diminution de la réponse et à une augmentation de la latence a été observé chez les Depleted. Une tendance a également été mise en évidence entre taille du cerveau et distance parcourue. Ces résultats suggèrent un impact potentiel de la carence

en DHA sur la capacité à échapper à un prédateur. Une analyse des AG des tissus et une mise en relation avec les variables de croissance et comportementale nous permettrait de répondre aux questions qui ont été ouvertes.

***Chapter 4 - The muscle biopsy to
study fatty acid composition in
sea bass***

Part 1: Research paper n°3 entitled “Non-lethal sampling method for the analysis of white muscle fatty acid profiles in European sea bass”

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1. Abstract

This study presents a novel non-lethal sampling method for assessing fatty acid (FA) composition in juvenile European sea bass (*Dicentrarchus labrax*) using subcutaneous white muscle biopsies. This research aimed to evaluate the suitability of the biopsy for FA analysis using two lipid extraction protocols and comparing them to a lethal routine method. The results showed that a mass of fresh tissue as low as 1.4 mg provided good quality FA chromatograms for both reserve and membrane lipids. Although the biopsy method displayed high variability in terms of FA quantity among intra-individual replicates, it showed good FA profile repeatability in both reserve and membrane lipids. The study highlights the potential of this non-lethal approach for studying FA dynamics in fish, with its application being particularly promising for ecological and experimental studies. However, careful biopsy implementation is recommended to account for potential lipid droplet and lipid distribution variability within the tissue.

Key words: Fatty acids, European sea bass, Muscle biopsy, Lipid composition, Method comparison

2. Introduction

Lipids are essential molecules in animals that serve both as energy sources and reserves (non-polar lipids; NL), as well as key structural components of cell membranes (polar lipids; PL) (Sargent et al. 2003). Due to their structural function, lipids modulate cell membrane functionality and the function of membrane proteins (Singer and Nicolson 1972; Hulbert and Else 1999; Tocher 2003). They also modulate cellular signalling and communication (Murphy 1990), gene expression, and act as hormones or intracellular messengers (for reviews see Sargent et al. (2003) and Tocher (2003)). They then modulate various biological processes in cells, tissues, organs, that ultimately affect the overall performance of an individual (Tocher 2010a). Both NL and PL are composed of fatty acids (FA), among which polyunsaturated fatty acids (PUFA) are the main component of cell membranes and thus play a key role in maintaining vital functions of most animals (Sargent et al. 2003). Among PUFA, eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA) have especially been demonstrated to be essential for most marine fish to ensure growth, reproduction, development (Tocher 2010). However, as for most animals, these FA are weakly synthesized *de novo* by fish, and must be obtained through the diet. In natural environments, EPA and DHA are primarily synthesized at the base of the aquatic food web by primary producers like marine microalgae (Arts and Kohler 2009b) and then accumulate through the food chain, ultimately reaching higher consumers (Sargent et al. 1993; Tocher et al. 2006; Li et al. 2010).

Fish fatty acid composition analysis is mandatory to meet several objectives, for example, to understand the link between dietary FA and physiological performance of an individual. Experimental research has explored for instance the links between dietary FA, fish muscle membrane FA composition, and individual performances, such as growth, locomotion or metabolism (Tocher 2003; Calder 2012). The effects of dietary FA on fish physiology have also been studied in the context of global change, in combination with other environmental stressors like hypoxia (McKenzie et al. 2000, 2008), warming (Gourtay et al. 2018; Vagner et al. 2019) or acidification (Hurst et al. 2019). Examining fish FA composition is also valuable for understanding the structure of trophic food webs in natural ecosystems, as some fatty acids serve as trophic biomarkers that can be used to identify consumer prey and elucidate trophic

interactions within an ecosystem (Dalsgaard et al. 2003; El-Sabaawi et al. 2010; Mathieu-Resuge et al. 2019; Bertrand et al. 2022).

Typically, in fish, FA analysis requires the sacrifice of the animal to obtain sufficient amount of tissue from specific organs (*e.g.*, 50-200 mg, wet weight WW, Meyer et al. (2017)) such as the liver, brain or muscle (Couturier et al. 2020; Betancor et al. 2021b) to meet the minimal biomass requirements according to the analytical protocol. Also, while FA content of some tissues can provide an integrated record of dietary intake over a period of weeks to months, monitoring changes in a fish FA composition at shorter time scale and over time becomes challenging, which could otherwise offer valuable insights into the dynamics of FA incorporation. Blood could be considered as an interesting tissue to monitor FA composition over time, but as the recommended volume that can be drawn from a live fish is from 0.1 to 10% (Lawrence et al. 2020), this would lead to a maximum blood volume of 0.2 mL, which might be insufficient for FA analysis. However, this is only the case for small animals like juvenile fish and larger animals can be sampled without risking killing the individual.

Non-lethal sampling methods have already been used in fish biology to address trophic ecology and ecotoxicology, using fins and scales (Cano-Rocabayera et al. 2015; Jeffries et al. 2021) or blood (Lawrence et al. 2020). Non-lethal sampling of muscle has also been realized to study mercury concentrations, with a tissue WW ranging from 47 to 220 mg from fishes of a minimal size of 36 cm and 600 g (Baker et al. 2004). In 2013, Olsen et al. studied muscle FA composition using biopsy on adult salmon *Salmo salar* with a mean size and weight of 84 cm and 6.6 kg, respectively, and with biopsy samples weighing a minimum of 40 mg WW. However, one of the main challenges of these non-lethal sampling methods is the necessity to take a small quantity of sampled tissue to guarantee minimal harm to fish, especially when working with small species or young individuals (Meyer et al. 2017). Given that lipid distribution within a tissue can exhibit heterogeneity (Nanton et al. 2007; Barbosa et al. 2018) the small amount of tissue collected through biopsies may lead to an inaccurate representation of the tissue's lipid content. This could result in a lack of repeatability, as the variability in FA composition between replicates obtained from different areas of the same tissue is likely to be high.

The objective of our research was to evaluate a novel nonlethal sampling technique for determining the FA composition of juvenile European sea bass (*Dicentrarchus labrax*; Linnaeus 1758) using subcutaneous white muscle biopsies. We anticipate that this approach will: i) yield adequate tissue sample quantities for generating interpretable FA profiles ii) would be repeatable within an individual, with a low intra-individual variability, also compared to the inter-individual variability in terms of FA quantity and proportions, and iii) produce results comparable to a traditional but lethal method of sampling using higher tissue biomass. As working with dry samples is of interest in some particular cases, such as field studies, thanks to its convenience for transport and storage (Sardenne et al. 2019), we wanted to test whether the conditioning (freeze-drying or fresh) of biopsied tissues had an effect on FA quantities and proportions and could be compared to our routine tissue analysis.

3. Material and Methods

3.1. Animal Ethics declaration

All fish manipulations were performed according to the Animal Care Committee of France (ACCF) and performed at the Ifremer facilities in Plouzané (France, Agreement number: B29-212-05). All fish manipulations were performed under anesthesia using tricaine (200 mg L⁻¹, ethyl 3-aminobenzoate methane sulfonate), and all efforts were made to minimize animal suffering during manipulation.

3.2. Animal origin

Juvenile European sea bass originating from aquaculture farm have been raised from January 2020 to April 2021 in a single tank. They were maintained at the natural seasonal temperature of the bay of Brest (mean = 14.5 ± 2.8; min-max: 10-19°C) and were fed *ad-libitum* with a commercial diet thrice a week (NeoStart, Le Gouessant, Lamballe, France, [Table S6](#)).

3.3. Muscle sampling and lipid extraction

In April 2021, six individuals randomly chosen (15 ± 2 cm; 50 ± 10 g) were euthanized using tricaine (400 mg L^{-1}) in seawater. On each individual, six subcutaneous white muscle biopsies were immediately performed on the left dorsal side of the fish, just behind the head, using a biopsy punch (2 mm LCH-PUK-20, Kai Medical, Solingen, Germany) (Figure S5). Samples were visually checked to be sure that only white muscle was sampled.

Three out of the six biopsies (further named “Fresh”, 8.8 ± 1.0 mg WW) were directly dived into 6 mL of a mix of chloroform/methanol (2:1, v/v), following a modified method from (Folch et al. 1957) for lipid extraction. The three other biopsies (further named “FD” for Freeze-Dried, 9.5 ± 0.8 mg WW) were first flash frozen in liquid nitrogen, and then freeze-dried for 36 hours before being dived into 6 mL of a mix of chloroform/ methanol (2:1, v/v).

After having performed biopsies, a large muscle sample (further named “MC” for Muscle control, 80.0 ± 24.4 mg WW) was sampled on the same area as where biopsies were performed, in each individual (Figure S5). MC were treated following our routine protocol for FA analyses. It was flash frozen, then grounded in liquid nitrogen into a homogenous powder and finally, 40 to 100 mg WW of muscle powder was dived in a 6 mL mix of chloroform/methanol (2:1, v/v). To ensure a total lipid extraction, all the lipid extracts (Fresh, FD, and MC) were sonicated for 10 min and agitated for 20 min before being stored at -20°C under nitrogenous atmosphere prior further FA analysis.

3.4. Analysis of the muscle fatty acid composition

3.4.1. Lipid separation

For all samples, lipids were separated into non-polar lipids (NL) and polar lipids (PL) fractions following the method described by Le Grand et al. (2014). An aliquot (one sixth) of the total lipid extract was used for Muscle control samples, whereas the whole lipid extract was used for biopsies (both Fresh and FD), as the mass of biopsy sampled tissue ranged from 1.4 to 14.4 mg for Fresh and from 5.5 to 13.6 mg for FD samples. 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 \times 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 methanol and collected in the same way as NL.

3.4.2. Non-polar lipid and polar lipid transesterification

NL and PL fractions were processed following a modified version of Mathieu-Resuge et al. (2019). Briefly, NL and PL fractions were evaporated to dryness under nitrogen. Fatty Acid Methyl Esters (FAME) were then formed by adding 800 μL of H_2SO_4 /methanol (3.4%, v/v) and heating 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 removal of the aqueous phase, the hexane phase, containing FAME, was washed two more times with hexane-saturated distilled water.

3.4.3. FAME analysis

Analyses of FAME was performed on a Varian CP8400 gas chromatograph using simultaneously two separations on a polar column (DBWAX: 30 m \times 0.25 mm ID \times 0.2 μm , Agilent) and an apolar column (DB5: 30 m \times 0.25 mm ID \times 0.2 μm , Agilent) using hydrogen as carrier gas with a flow rate set at 2 mL.min⁻¹, in splitless mode. The oven temperature was programmed as follows: first, initial heating from 60°C to 150 °C at 50 °C.min⁻¹, then to 170 °C at 3.5 °C.min⁻¹, to 185 °C at 1.5 °C.min⁻¹, to 225 at 2.4 °C.min⁻¹ and finally to 250 °C at 5.5 °C.min⁻¹ and maintained for 25 min. Injector temperature was set at 250°C, detector temperature at 280°C. Identification of FAME was realized by comparison of their retention time based on 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 ($\mu\text{g}\cdot\text{mg}^{-1}$ WW). Fatty acid proportion was defined as the mass percentage of each fatty acid to the total fatty acid content.

3.5. Statistical analysis

All statistical analyses were conducted using R Studio (v4.2.1). In order to determine if the biopsy method would be spatially repeatable within one individual, we calculated the intra-individual variation as the CV (standard deviation/mean) of the studied parameter (FA quantities and proportion), for each individual and for each biopsy method (n=3 Fresh and n=3 FD for each individual). This was performed on both quantity and proportions of EPA, DHA, PUFA, monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) in both NL and PL fractions. In order to be able to characterize the range of intra-individual variability, we compared these intra-individual CV to the corresponding inter-individual CV performed on MC samples (n=6).

We hypothesized that if the intra-individual CV exceeded either 10% (Sardenne et al. 2019) or the corresponding inter-individual CV, the biopsy method would not be repeatable enough to be validated and used with confidence. On the contrary, if the intra-individual variability was lower than 10% or than the inter-individual variability, we hypothesized that the biopsy method would be repeatable. It is the necessary condition allowing us to calculate the mean of the biopsy triplicates and to conduct a PERMANOVA test to compare the effect of the method (Fresh, FD, MC) on the FA proportions. We used the PERMANOVA to test the general profile of FA with “Method” (i.e. Fresh, Freeze-dried, and Control), as a factor and each method was set as fixed level. Only the ten main FA were considered in this analysis. To evaluate if there were any differences in specific FA proportions between the methods, each FA was analyzed using a one-way ANOVA test. Normality and homoscedasticity were assessed on residuals with the Shapiro-Wilk and Bartlett tests, respectively. If ANOVA assumptions were not met, a Kruskal-Wallis test was employed on not-transformed data (Hecke 2012). When ANOVA or Kruskal-Wallis tests indicated significant differences, a post-hoc Tukey HSD test was conducted to differentiate the conditions. Data are presented as mean \pm SEM.

4. Results

4.1. Adequacy of biopsied tissues for FA analysis

White muscle biopsy samples ranging from 1.5 to 14.4 mg were sufficient to obtain good quality chromatograms. In addition, the biopsy method allowed us to quantify over 90% of the FA identified in the Muscle control method. The biopsy total lipid extracts contained $86.1 \pm 13.5 \mu\text{g}$ (min-max: 5.5-409.0 μg) of FA for NL and $28.8 \pm 1.7 \mu\text{g}$ (min-max: 10.8-45.6 μg) of FA for PL (not shown). These values were similar to those contained in the 1/6 aliquots of MC total lipid extracts used in the routine method: $86.1 \pm 13.5 \mu\text{g}$ (min-max: 5.5-409.0 μg) of FA for NL and $28.8 \pm 1.7 \mu\text{g}$ (min-max: 10.8-45.6 μg) of FA for PL (not shown).

4.2. Repeatability of the biopsy method within an individual

Our results indicate that both biopsy methods resulted in high intra-individual variability in total FA quantity in NL (**Figure 44A**) (Fresh: min-max: 1.0-98.4 $\text{mg}\cdot\text{g}^{-1}$, FD: min-max: 0.7-74.4 $\text{mg}\cdot\text{g}^{-1}$) this variability, illustrated by the coefficient of variation of the three replicates per individual and biopsy method (CV mean = $75 \pm 40\%$, min-max = 15-147%) was always above 10% (**Figure 44**). The CV values were higher than those of MC for 5 out of 6 individuals for both methods. In PL, the intra-individual variability of total FA quantity was lower than in NL (**Figure 44**) but still displayed considerable differences between extreme values (Fresh: min-max: 2.2-17.7 $\text{mg}\cdot\text{g}^{-1}$, FD: min-max: 1.4-5.6 $\text{mg}\cdot\text{g}^{-1}$). The CV was higher than 10% for most individuals and ranged from 3% to 74% for both Fresh and FD methods (CV mean = $23 \pm 19.8\%$, **Figure 44**). The CV values were higher than those of MC for 4 out of 6 individuals for both methods. In general, no biopsy method distinguished itself from the other in terms of variability.

Regarding the NL fraction, SFA, MUFA and PUFA proportions (**Figure 45A**) varied less within individuals (CV <10%), than EPA and DHA (**Figure 45**, EPA min-max: 3-21.1%; DHA min-max: 2.9-49.6%), whatever the sampling method employed, either biopsy or routine. For EPA, the biopsy CV was always lower than the MC CV. For DHA, 11 out of 12 CV were lower than the MC CV. Regarding PL, even if the CV varied among individuals, they remained under 10% for all individuals and for both methods (**Figure 45B**).

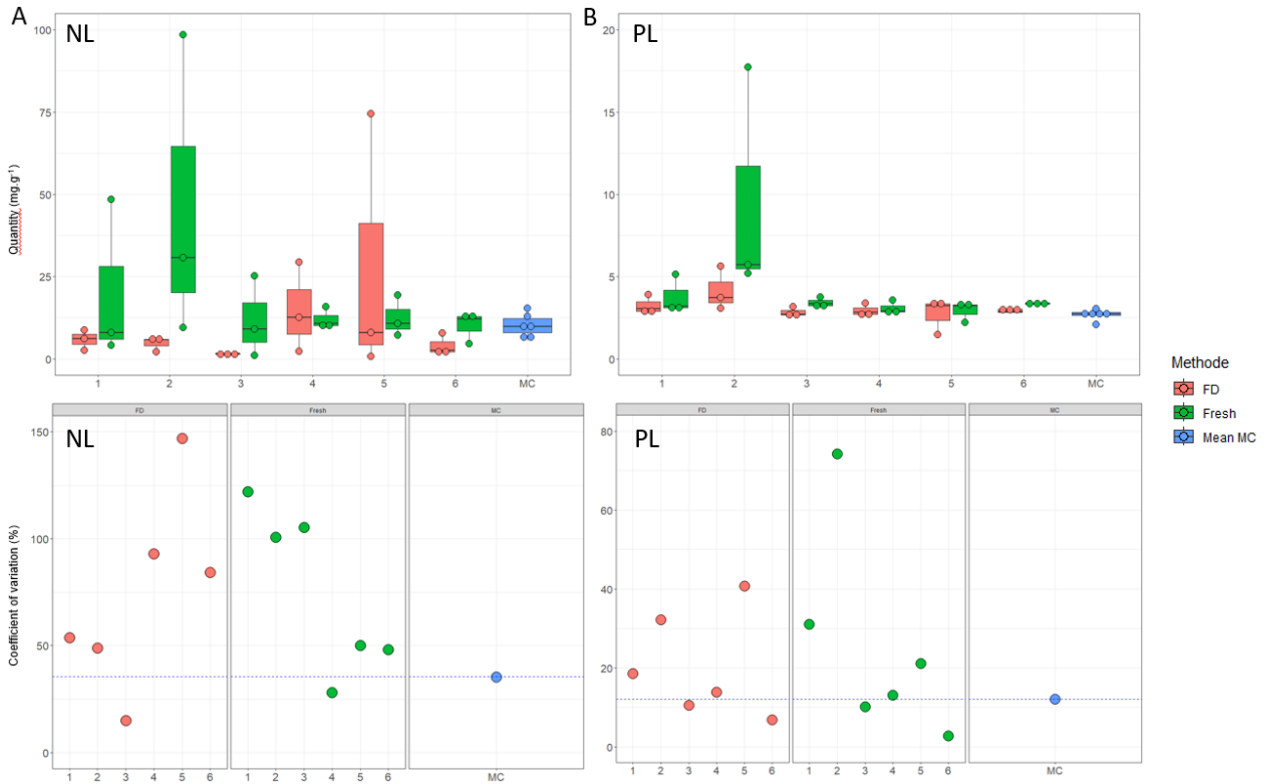


Figure 44: Fatty acid quantities (mg.g⁻¹ WW upper panels) and coefficients of variation (percentage, lower panels) of FA quantities in non-polar lipids (A, C) and polar lipids (B, D) of different method. The different methods are presented as followed: Freeze-dried protocol (FD, red, n=3 intra-individual replicates), fresh protocol (Fresh, green, n=3 intra-individual replicates) and muscle control (MC, blue, n=6 inter-individual replicates) in white muscle of juvenile European sea bass, *Dicentrarchus labrax*. On the boxplots, each dot represent a replicate. On the dot plot, each dot represent the CV of the 3 (for biopsies) or 6 replicates (for MC) and the blue dotted line represent the value of MC CV.

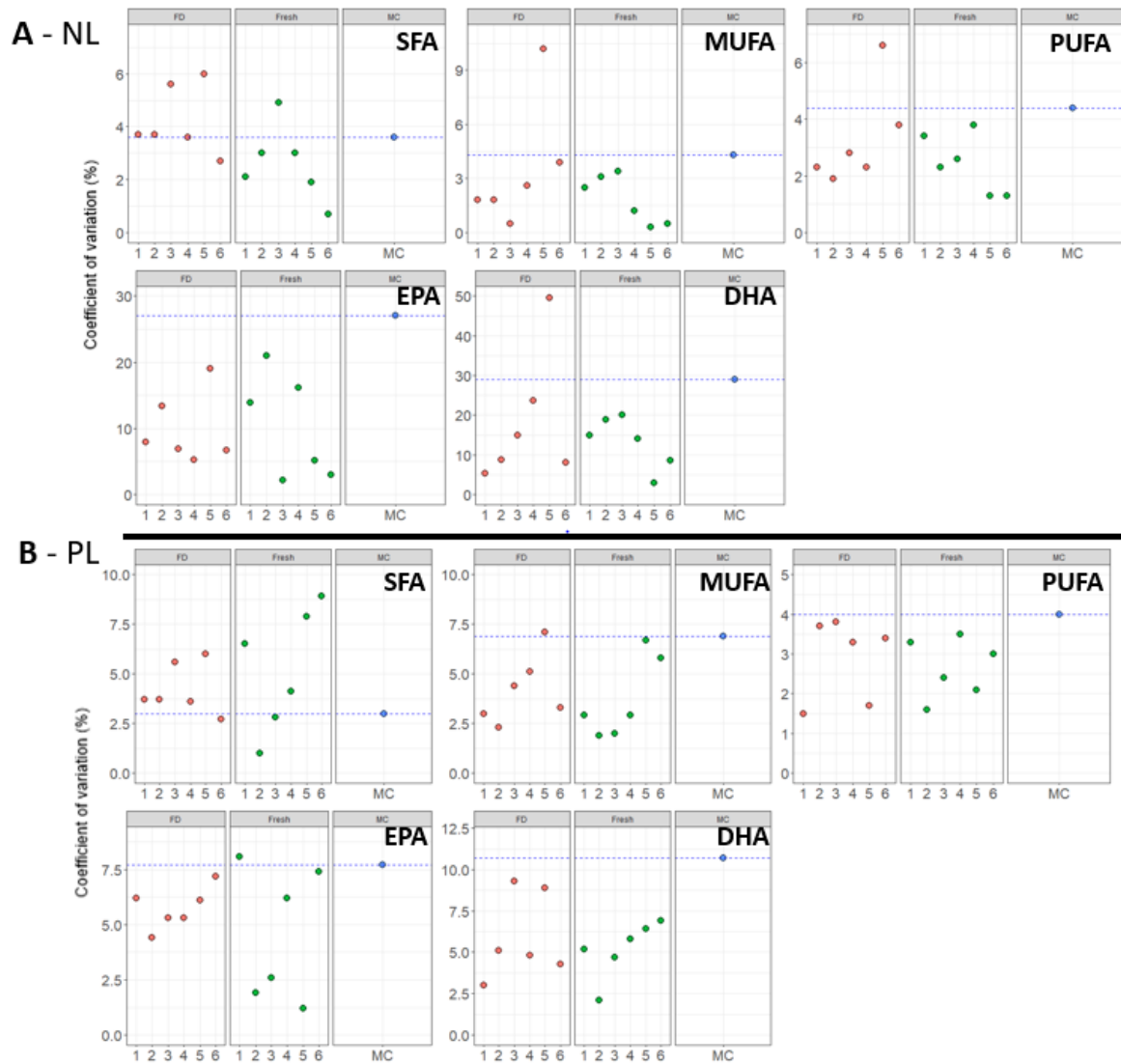


Figure 45: Coefficient of variation (percentage) of FA proportions in NL (A) and PL (B) for the different methods. Main family and FA are presented: Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for freeze-dried protocol (FD, red, n=3 intra-individual replicates), fresh protocol (Fresh, green, n=3 intra-individual replicates) and muscle control (MC, blue, n=6, inter-individual replicates) in white muscle of juvenile European sea bass, *Dicentrarchus labrax*. Each dot represent the CV of the 3 (for biopsies) or 6 replicates (for MC) and the blue dotted line represent the value of MC CV.

4.3. Comparison between biopsy methods and a traditional but lethal method of sampling

Concerning FA quantities, the biopsy results showed a high intra-individual variability for both methods. We then considered that biopsy replicates would not be repeatable enough and then could not be pooled to obtain a mean per individual, and thus, that testing the effect of method on FA quantities would not be relevant.

Regarding biopsy methods, most of the FA proportions in NL and PL had a CV <10% and had lower CV than the inter-individual one (Figure 45). We thus hypothesized that the biopsy triplicates performed within individuals could be pooled, and that the effect of method can be tested on FA proportions. When considering the 10 major fatty acids (Figure S6), no significant effect of the method was observed for both NL and PL (PERMANOVA, $p > 0.05$). However, when considering the FA individually, 16:0 and 18:0 FA exhibited a significantly higher proportion in the NL fraction when analyzed with the FD method than with the two others (Table 20), and consequently, SFA proportions followed this trend. No statistical differences were observed among the three methods for PL FA proportions (Table 20).

Table 20: Fatty acid proportion (mass percentage of each FA over total fatty acids) of the 10 major fatty acids measured in non-polar (NL) and polar (PL) lipids with the three methods: Fresh biopsy, freeze-dried biopsy (FD) and muscle control (MC) in white muscle of juvenile European sea bass, *Dicentrarchus labrax*. Data are presented as inter-individual means (\pm SEM, $n=6$), after pooling intra-individual biopsy triplicates. The effect of the method on the FA proportions was tested using a one-way ANOVA or Kruskal-Wallis test, followed by Tukey's post hoc test or pairwise Wilcoxon test. Significance was accepted at $p < 0.05$. Different letters read in line indicate significant differences between methods. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, - NS (not significant). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

NL Fatty Acid	Muscle Biopsies			Statistics
	Fresh	Freeze-Dried	Muscle Control	
14:0	2.9 \pm 0.2	3.1 \pm 0.2	3.1 \pm 0.2	-
16:0	18.1 \pm 0.3 ^a	18.2 \pm 0.1 ^a	18.2 \pm 0.1 ^a	**
18:0	3.7 \pm 0.2 ^a	3.6 \pm 0.1 ^a	3.6 \pm 0.1 ^a	**

16:1n-7	5.7 ± 0.3	5.9 ± 0.3	5.9 ± 0.3	-
18:1n-7	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	-
18:1n-9	29.0 ± 1.5	28.8 ± 1.2	28.8 ± 1.2	-
18:2n-6	13.4 ± 1.1	13.1 ± 0.7	13.1 ± 0.7	-
18:3n-3	2.5 ± 0.2	2.4 ± 0.1	2.4 ± 0.1	-
20:5n-3	5.5 ± 0.7	5.5 ± 0.6	5.5 ± 0.6	-
22:6n-3	2.8 ± 0.4	2.8 ± 0.3	2.8 ± 0.3	-
SFA	25.6 ± 0.6 ^a	26.0 ± 0.3 ^a	26.0 ± 0.3 ^a	**
MUFA	42.8 ± 0.9	43.1 ± 0.8	43.1 ± 0.8	-
PUFA	31.3 ± 0.5	30.7 ± 0.5	30.7 ± 0.5	-

PL Fatty Acid	Muscle Biopsies		Muscle control	Statistics
	Fresh	Freeze-Dried		
16:0	23.2 ± 0.3	23.7 ± 0.5	22.6 ± 0.3	-
18:0	5.9 ± 0.1	5.8 ± 0.1	6.1 ± 0.2	-
16:1n-7	2.5 ± 0.1	2.4 ± 0.1	2.7 ± 0.2	-
18:1n-7	2.1 ± 0.0	2.1 ± 0.0	2.1 ± 0.1	-
18:1n-9	13.1 ± 0.4	13.6 ± 0.4	14.0 ± 0.5	-
18:2n-6	5.8 ± 0.4	5.9 ± 0.4	6.2 ± 0.4	-
20:4n-6	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.1	-

20:5n-3	14.7 ± 0.2	14.5 ± 0.2	14.2 ± 0.4	-
22:5n-3	2.4 ± 0.1	2.2 ± 0.2	2.3 ± 0.1	-
22:6n-3	17.0 ± 0.5	16.8 ± 0.5	16.3 ± 0.7	-
SFA	31.1 ± 0.4	31.5 ± 0.5	30.6 ± 0.4	-
MUFA	19.8 ± 0.5	20.2 ± 0.5	21.1 ± 0.6	-
PUFA	46.8 ± 0.5	46.2 ± 0.5	45.8 ± 0.7	-

5. Discussion

5.1. Adequacy of the biopsied tissue for FA analysis

Our results show that white muscle biopsy using punch of 2 mm on sea bass juvenile of about 15 cm and 50 g is adequate to perform fatty acid analysis. The sampled biopsied tissues were sufficient to yield at least 5.5 µg of FA for NL and 10.8 µg for PL, which was enough to obtain qualitative chromatograms with FA having a peak area reaching the minimal threshold. The average FA quantity extracted was even much higher than these minimums, which supports that white muscle biopsy method provides an adequate quantity of tissue for FA analysis. Olsen et al. (2013) stated that 5 µg of FA is the minimum amount required to obtain interpretable chromatograms. In the same study, the authors suggested to use a minimum of 60 mg WW of muscle but this value should consider the type of tissue, life stage of the fish, the species, as well as the analytical equipment used. The fish size and weight are crucial considerations when sampling tissue to ensure minimal impact on the animal. In our study, we did not tested the effect of biopsy on fish growth and health condition, as they were sacrificed. But in a related study, Quéméneur et al. (2022) conducted red muscle biopsies on European sea bass of a similar size (around 76.7g in weight) using the same biopsy punch as ours. They observed that the biopsy did not affect the fish's immune system, and the fish continued to gain weight post-procedure. Henderson et al. (2016) used a larger biopsy punch (4mm compared to our 2mm) on Moses perch *Lutjanus russelli* and Grass emperor *Lethrinus*

laticaudis. These fish had a total length close to those used in our study (approximately 15.8 and 14.0 cm, respectively), and it was found that the biopsy procedure led to only temporary stress and did not result in any fish mortality.

5.2. How repeatable is the FA analysis within an individual using the biopsy method?

Quantities of FA obtained with the biopsy methods were highly variable within individuals for both NL (almost 100-time fold between min and max) and PL (almost 5-time fold between min and max). One hypothesis could be that these variations might be caused by the presence of lipid droplets in the tissue. Lipid droplets are intracellular structures mainly composed of NL, serving as lipid storage in various tissues, including muscle (Favé et al. 2004; Kiessling et al. 2006; Granneman et al. 2017) that would hence induce higher FA content variability in NL rather than in PL. However, lipid droplets are less abundant in fish white muscle compared to red muscle (Kiessling et al. 2006; Granneman et al. 2017) and may only be present in few samples, leading to FA quantities significantly higher in samples containing lipid droplets. In addition, the distribution of lipids in white muscle is known to vary depending on the sampling location (Charette et al. 2021) which may cause FA quantities to differ based on the sampling location of the biopsy.

5.3. Comparison between methods and traditional lethal sampling

Contrary to quantities, the analysis of FA proportions using biopsy was repeatable. The low variability for SFA, MUFA, and PUFA proportions in NL measured within individuals (CV<10%) suggests relative homogeneity of these proportions within individuals, while higher variability in EPA and DHA proportions could indicate dietary or metabolic impacts leading to specific heterogeneity in FA profile. Is the biopsy method comparable to a traditional but lethal method.

For NL, the variability measured within individuals and illustrated by the high CV values that exceeded the targeted 10% threshold as well as the inter-individual variability (Sardenne et al. 2019), makes it difficult to compare the two-biopsy methods with the control methods. A higher quantity of tissue would have supported less variability between methods, as shown

by Sardenne et al. (2019) who compared the effect of extraction method from wet and dry muscle tissue (around 100 mg) from gilthead seabream *Sparus aurata* and did not find any differences between methods when considering total lipids (NL + PL).

In terms of proportions, the biopsy method seemed to yield results comparable to the MC for both extraction protocols. As the intra-individual variability did not reach the threshold of our study, we considered the biopsy method repeatable and comparable for both extraction protocols. Indeed, no differences between biopsy and control methods were observed when considering the whole profile of 10 major FA for NL and PL. However, differences were observed when considering individually certain SFA (16:0 and 18:0) for the FD method despite being repeatable with our criteria. Nevertheless, we acknowledge that applying a uniform threshold to both proportions and quantities may not be wholly pertinent, as it involves two distinct aspects. It might be necessary to calibrate this threshold more appropriately, depending on whether we are investigating proportions or quantities due to their different nature and analytical variabilities.

6. Conclusion

This study assessed the feasibility of a novel approach for measuring FA composition in European sea bass using subcutaneous white muscle biopsies. The method allowed successfully obtaining adequate sample biomass and produced high-quality chromatograms for both analytical protocols. FA quantity obtained with both biopsy methods was highly variable within individuals, potentially due to factors such as lipid droplets and heterogeneous lipid distribution in the sampled tissue. We thus recommend to take a special care when realizing the biopsy, and to ensure that a sufficiently deep piece of tissue is removed, avoiding the muscle directly in contact with the skin which has different FA composition. The analysis of FA proportions appeared to be achievable with biopsy with both protocols. Although it is challenging to determine the optimal biopsy method for FA analysis in fish, this study demonstrates the potential of using subcutaneous white muscle biopsies in European sea bass for examining FA composition, with a particular focus on FA proportions. The fish species, its fat content and its size must also be considered to optimize lipid extraction and minimize the effect of the procedure on the animal.

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Competing interests

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper. We hereby disclose that Dr. David Mazurais, a contributing author of this article, also serves as an Editor on Fish Physiology and Biochemistry board, and affirm that all review and acceptance processes for this publication were conducted impartially and without bias.

Supplementary Materials – Chapter 4

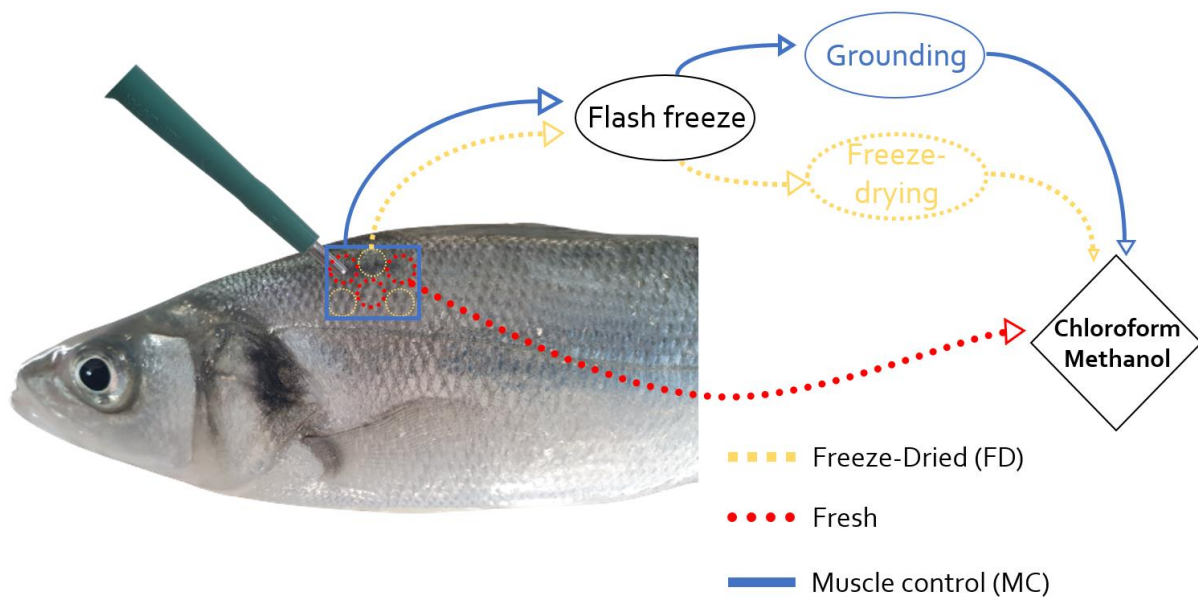


Figure S5: Sampling protocol performed on six individuals of European seabass (*Dicentrarchus labrax*). Biopsies are represented by the circles (n=6 biopsies per individual). Fresh method is represented by the red dotted line (n =3 per individual, dived directly in chloroform/methanol (2:1, v/v)). Freeze-dried method is represented by the yellow dotted line (n=3 per individual, flash frozen then freeze-dried and dived into chloroform/methanol (2:1, v/v)). On all individuals, the rest of the muscle tissue was sampled and is represented by the blue solid line (flash frozen then grounded in liquid nitrogen and dived in chloroform/methanol (2:1, v/v)).

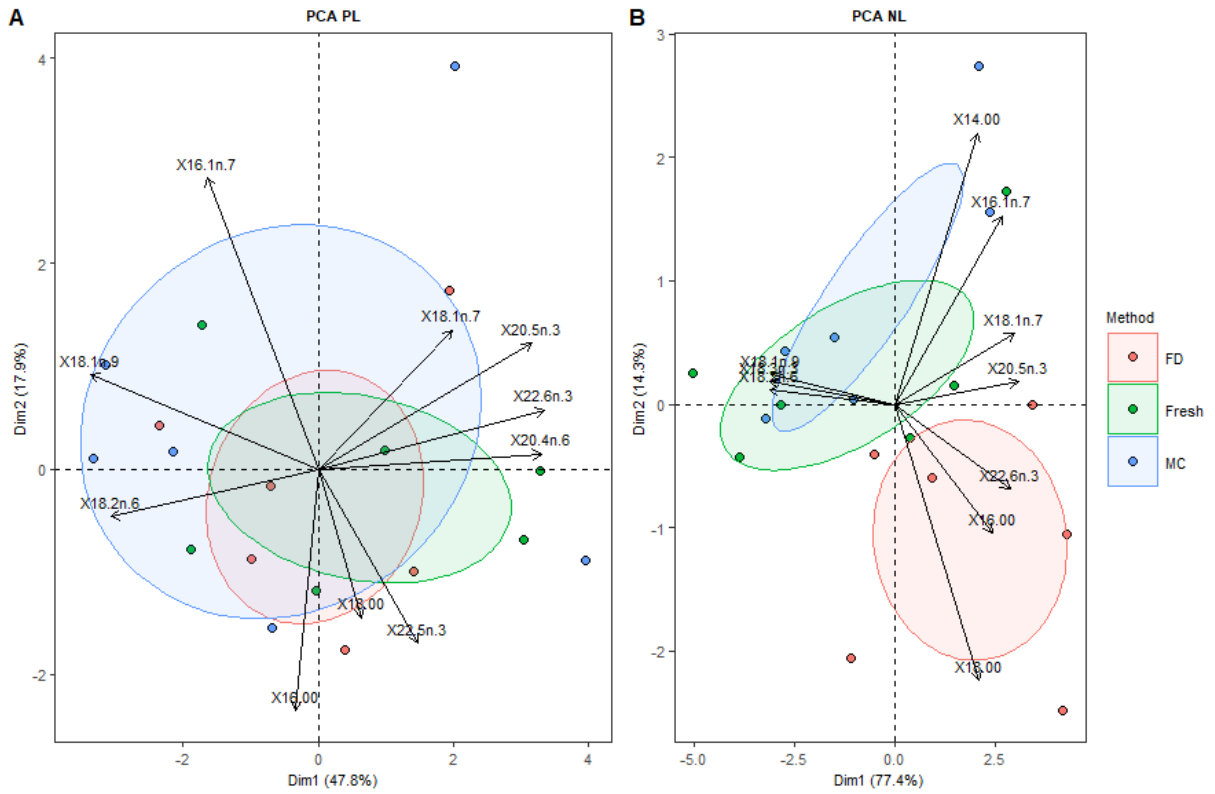


Figure S6: Principal Component Analysis (PCA) of the ten most abundant fatty acids (FA) for polar lipids (PL, A) and neutral lipids (NL, B) for the different methods. Red dots represent a pooled biopsy triplicate for the Freeze-dried method (n=6). Green dots represent a pooled biopsy triplicate for the Fresh method (n=6). Blue dots represent the Muscle Control (MC) method (n=6). All ellipses represent confidence ellipses.

Table S6 : Fatty acid composition of the diet. Values are expressed as percentage of total FA. Data are presented as mean \pm SD (n=3). SFA: Saturated Fatty Acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

FA	Mean \pm sd
14:0	8.31 \pm 0.23
16:0	23.06 \pm 0.17
18:0	4.60 \pm 0.02
Σ SFA	37.93 \pm 0.38
16 :1n-7	9.50 \pm 0.11
18 :1n-7	3.85 \pm 0.03
18 :1n-9	13.05 \pm 0.08
20 :1n-9	3.36 \pm 0.09
22 :1n-11	3.78 \pm 0.07
Σ MUFA	36.21 \pm 0.21
16 :4n-1	1.42 \pm 0.01
18 :2n-6	4.84 \pm 0.04
18 :4n-3	1.26 \pm 0.01
20 :4n-6	0.59 \pm 0.00
20 :5n-3	7.49 \pm 0.06
22 :6n-3	3.04 \pm 0.05
Σ PUFA	25.50 \pm 0.18
Σ n-6	6.17 \pm 0.07
Σ n-3	14.74 \pm 0.12

Part 2: Research paper n° 5 entitled “White muscle biopsy to monitor FA composition of European sea bass submitted to a dietary conditioning”

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In preparation for Fish physiology and biochemistry

1. Abstract

This study aimed to assess the impact of dietary changes on the fatty acid (FA) composition in the white muscle of European sea bass and explored the potential of muscle biopsy as a tool to track these changes. Results confirmed that muscle biopsy effectively monitors FA shifts without adversely affecting fish growth. Notable findings included a relative stability in phospholipid (PL) quantities and proportions post-dietary modification, emphasizing distinct metabolic pathways for FA in cellular membranes. Moreover, significant variations were observed in monounsaturated fatty acid (MUFA) levels between recently captured fish (October) and those kept under experimental conditions (February), with October fish displaying resilience and adaptation to new dietary regimes. Overall, the study underscores the efficacy of muscle biopsy in understanding dietary impacts on aquatic species and the robust adaptability of European sea bass following diet changes.

2. Introduction

Fatty acids (FA) are essential hydrocarbon chains that play crucial roles in the structural and functional aspects of living organisms (Sargent et al., 2003). Being primary constituents of cell membranes as phospholipids (or Polar Lipids, PL), the type and proportion of FA determine the fluidity, permeability, and overall functionality of these biological barriers (Singer & Nicolson, 1972). Beyond their structural roles, FA are pivotal for energy storage. Triacylglycerol (or Neutral Lipids, NL) serve as the principal energy reservoirs in many

organisms (Sargent et al., 2003). Furthermore, certain long-chain polyunsaturated fatty acids (LC PUFA), particularly eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid (ARA) are precursors for molecules involved in inflammation, immunity, and various signaling pathways (Calder, 2006). EPA and DHA are particularly critical for neural function, normal behavior and prevention of diseases (Lauritzen et al., 2001; Simopoulos, 2002). To understand the roles and dynamics of FA in physiological, ecological, and nutritional contexts, monitoring the FA composition in fish is important. The FA profile can serve as a direct reflection of dietary intake, offering insights into the nutritional status and health of the organism (Sargent et al., 1995). Changes in the dietary source or quality can lead to alterations in tissue FA composition, which can affect various physiological processes (Bou et al., 2017). In addition, the use of FA biomarkers has emerged as a powerful tool in recent decades to improve our understanding of trophic interactions (Iverson et al., 2004). These biomarkers, once incorporated into consumers, can be used to trace dietary sources and identify trophic linkages (Dalsgaard et al., 2003). FA profiles can indicate shifts in primary producer communities due to environmental perturbations or anthropogenic influences, thereby serving as early warning signals of ecosystem change (Budge et al., 2006).

With growing awareness of animal welfare and the ethical treatment of research subjects, there is an increasing emphasis on minimizing harm. Non-lethal methods align with this, ensuring that organisms are not sacrificed for the sake of data collection. These techniques have previously been used in fish to study mercury concentration or stable isotopes signature (Cano-Rocabayera et al. 2015; Smith et al., 2015). Among them, the muscle biopsy has proven to be safe (no impact on survival rate, growth nor immune system) for fish (Henderson et al., 2016; Quéméneur et al., 2022). In addition, we previously demonstrated that it was possible to obtain sufficient amounts of tissue and that it was relevant to measure FA proportions in the European sea bass using biopsy method (Peron et al., in review). However, it is not known yet if the muscle biopsy can be used to effectively monitor a changing FA composition over time and requires further experimental validations. In this context, the aim of this study was to assess if the white muscle biopsy allowed following the FA composition of fish after a nutritional change.

3. *Material and Methods*

3.1. Ethical statement

All of the experiments were performed with respect to 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.

3.2. Animals

3.2.1. Collection

About 150 juvenile European sea bass were captured in the Seine estuary during the NOURDEM survey in August 2021 (Le Goff et al, 2019). 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.

3.2.2. Rearing and experimental diets

Fish were acclimated in quarantine during 66 days in a single 2000 L tank with open circuit and natural temperature following the natural variation of the bay of Brest. During 30 days, fish were fed a mix (from 25 to 100%) of frozen seafood and commercial pellets (NeoStart, Le Gouessant, Lamballe, France; Table 1) in order to acclimate them to pellets. After these 30 days, fish were fed 100% of the commercial diet for 36 days.

After a total of 66 days in 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) in open circuit, exposed to a 12h light / 12h dark photoperiod cycle, and natural temperature ($14.4 \pm 2.8^\circ\text{C}$). Before being transported to their tank, 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), and underwent a white muscle biopsy (2021 October) following the Fresh protocol described in Peron et al. (in review, part 1 of the present Chapter).

Fish were then progressively fed with an experimental diet differing from the commercial one (from 25 to 100% of the experimental diet in 2 weeks; [Table 21](#)). The experimental diet was manufactured at INRAE (Donzacq, France). After 130 days of nutritional conditioning with the experimental diet, fish underwent a second white muscle biopsy (2022 February).

Table 21: Fatty acid composition of the commercial and experimental diets. Data are expressed as mean \pm standard deviation (n=3).

AG	2021 October	2022 February
14:0	8.3 \pm 0.0	5.9 \pm 0.0
16:0	23.1 \pm 0.2	15.1 \pm 0.1
18:0	4.6 \pm 0.0	3.6 \pm 0.0
Σ SFA	37.9 \pm 0.4	25.9 \pm 0.2
16 :1n-7	9.5 \pm 0.1	6.1 \pm 0.0
18 :1n-7	3.8 \pm 0.0	3.0 \pm 0.0
18 :1n-9	13.1 \pm 0.1	17.8 \pm 0.2
Σ MUFA	36.2 \pm 0.2	32.6 \pm 0.1
18 :2n-6	4.8 \pm 0.0	4.9 \pm 0.1
20 :4n-6	0.6 \pm 0.0	0.9 \pm 0.0
20 :5n-3	7.5 \pm 0.1	14.4 \pm 0.2
22 :5n-3	0.9 \pm 0.0	1.9 \pm 0.0
22 :6n-3	3.0 \pm 0.1	7.7 \pm 0.1
Σ PUFA	25.5 \pm 0.2	40.5 \pm 0.3

3.3. Growth parameters

Eighteen fish (three from each of the six tanks) were used for this study. Their growth and length was measured on the date of the biopsies (14 Oct. 21 and 21 Feb. 22). These data allowed to calculate the Fulton's condition factor (CF, arbitrary units):

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

Where W is the weight (g) and TL the Total Length (cm).

The growth rate was assessed by calculating the Thermal Growth coefficient:

$$TGC = 1000 \times \left(\frac{W_{final}^{1/3} - W_{initial}^{1/3}}{T \times D} \right)$$

Where W_{final} is the weight at the end of the experiment, $W_{initial}$ is the weight at the beginning of the experiment, T is the average temperature during the experiment (12.5°C) and D is the number of days between the first and the second biopsy (130 days).

3.4. Sample analysis

3.4.1. Biopsy samples

The muscle tissues of three fish from each of the six tanks were analysed (n=18) for both biopsy samples (October, 6.3 ± 2.4 mg and February, 5.9 ± 3.1 mg).

3.4.2. Fatty acid analysis

The fatty acid analysis was performed as detailed in Peron et al. (in review) for the Fresh tissues (part 1 of this Chapter). The FA analysis of the diets was performed as detailed in General Material and Methods (section 4.1).

3.5. Statistical analysis

All statistical analyses were conducted on RStudio (V.4.2.3). For the FA composition of both biopsies, differences were evaluated using a linear mixed model (package *lme4*, function *lmer*). Each FA (e.g DHA) was the dependent variable. The date of the biopsy was the fixed-effect explanatory variable (two levels; 2021 October or 2022 February). The individual was considered as a random effect (18 levels, one for each individual). The final model was tested with a t-test through the package *lmerTest*. The correlation between NL proportions and CF were tested using Spearman's correlation. Significance was accepted at $p < 0.05$. All data are presented as mean \pm standard deviation. A principal component analysis (PCA) was performed to visualize the dispersion of the FA between both fractions (NL and PL) and biopsy (2021 October and 2022 February).

4. Results

4.1.1. Growth parameters

Between October and February, fish weight, length and condition factor significantly increased. The fish displayed a positive growth rate (TGC) throughout the experiment ([Table 22](#)).

Table 22: Fish growth and condition parameters. W: Weight (g), TL: Total Length (cm), TGC: Thermal Growth Coefficient (g degree days⁻¹), CF: Fulton's condition factor

Parameter	2021 October	2022 February	Statistics
Weight	64.5 ± 22.1	97.7 ± 30.8	< 0.001
TL	18.5 ± 2.3	20.1 ± 2.1	<0.001
TGC	-	0.4 ± 0.2	-
CF	1.0 ± 0.1	1.2 ± 0.1	0.002

In both NL and PL, the quantity of FA in muscle was much higher after fish were fed the experimental diet (**Figure 46**). The percentage of NL was positively correlated with the condition factor in October 2021 ($p < 0.05$) and showed a non-significant trend in February ($p = 0.13$) (**Figure 47A, B**).

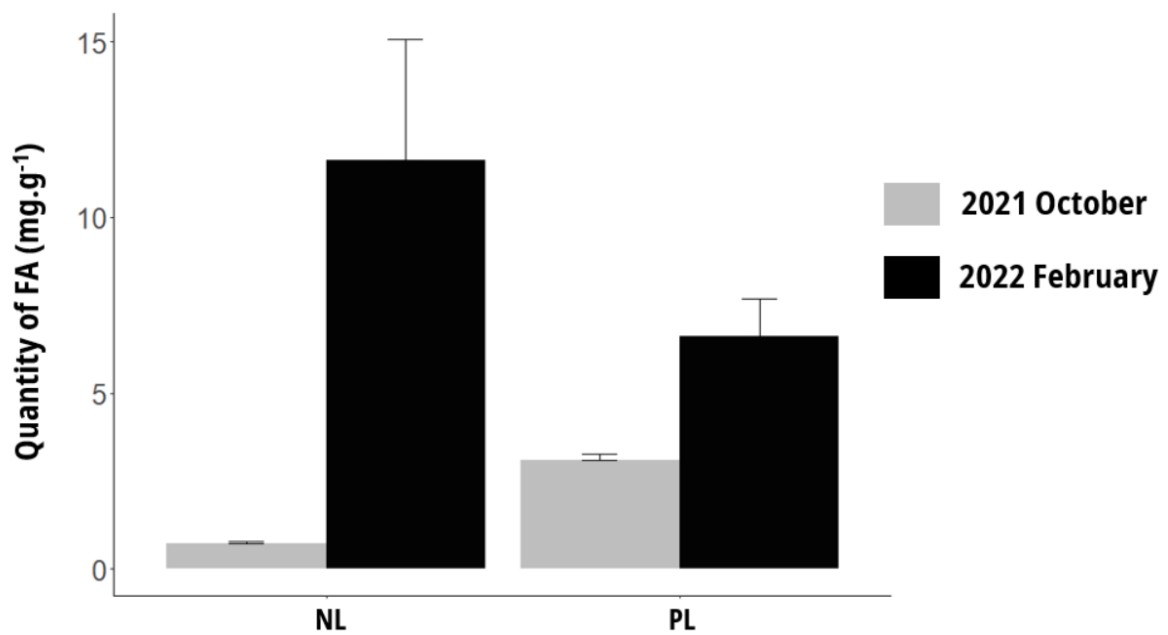


Figure 46: Quantity of total FA in NL and PL (mg.g⁻¹). The red bars represent the fish fed the commercial diet during 66 days (biopsies performed in October 2021) and the blue bars represent the same fish after they were fed an experimental diet for 130 days (biopsies performed in February 2022).

4.1.2. Fatty acid composition

In NL, the proportions of SFA decreased from October 2021 to February 2022 mostly due to differences in 16:0 and 18:0. This reflected the composition of the diets as the commercial diet (October 2021) contained much more SFA than the experimental diet (38 vs 28%,

respectively). On the contrary, proportions of MUFA were much higher in February compared to October and all MUFA (16:1n-7; 18:1n-7 and 18:1n-9) were higher in February but that was not a reflection of the diet as it contained less MUFA in February 2022. PUFA remained stable between October and February. Notably, EPA (20:5n-3) was higher in February compared to October (**Figure 48A, Table S7**).

In PL, the overall proportions for each family of FA (SFA, MUFA, and PUFA) was more stable than in NL. However, they displayed the same pattern as in NL with SFA and PUFA being higher in October, compared to February and MUFA being higher in October compared to February (**Figure 48B, Table S8**).

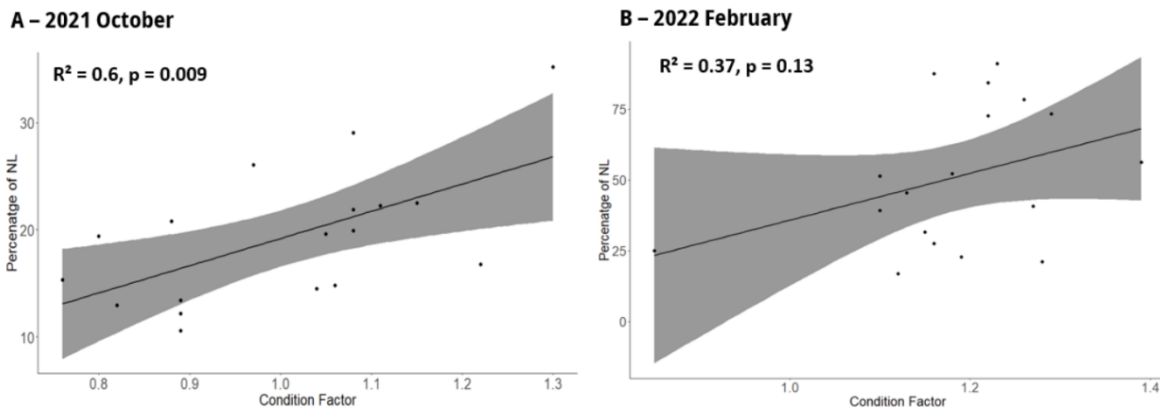


Figure 47: Spearman correlation between the condition factor and the percentage of NL in fish muscle obtained from biopsy in 2021 October (A, after 66 days of commercial diet) and in 2022 February (B, after 130 days of experimental diet).

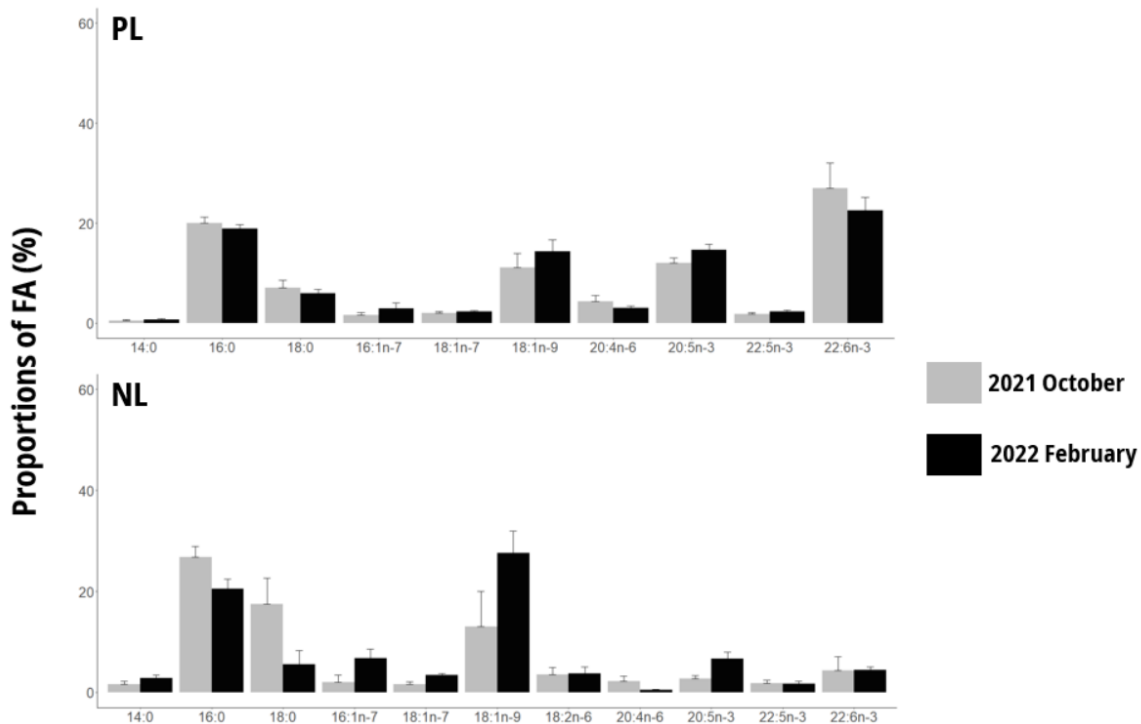


Figure 48: Fatty acid proportions in Neutral Lipids (A) and Polar lipids (B). Only FA that were above 0.5% and detected in all samples are represented. Red bars represent 2021 October (n=18) and blue bars represent 2022 February (n=18).

The inertia of major FA in PL and NL is represented with a PCA (**Figure 49**). The results show a clear distinction between NL and PL FA for both biopsies. The PL FA proportions for both biopsies are correlated with PUFA and NL proportions are correlated with SFA and MUFA.

However, we observed that, depending on the period of biopsy, fish NL and PL contents were correlated with different fatty acids. After being fed the commercial diet (2021 October), fish NL content was correlated with SFA and particularly 14:0 and 16:0, while it was correlated with MUFA and particularly 16:1n-7 and 18:1n-7 after fish were fed the experimental diet (February 2022) (**Figure 49**).

After being fed the commercial diet (2021 October) fish PL content was more particularly correlated with arachidonic acid (ARA; 20:4n-6), while it was more correlated with EPA (20:5n-3) and DHA (22:6n-3) after fish were fed the experimental diet (2022 February) (**Figure 49**). In addition, in October 2022, the individual variation between fish was stronger for both NL and PL as it is highlighted by a more widespread distribution of dots (**Figure 49**).

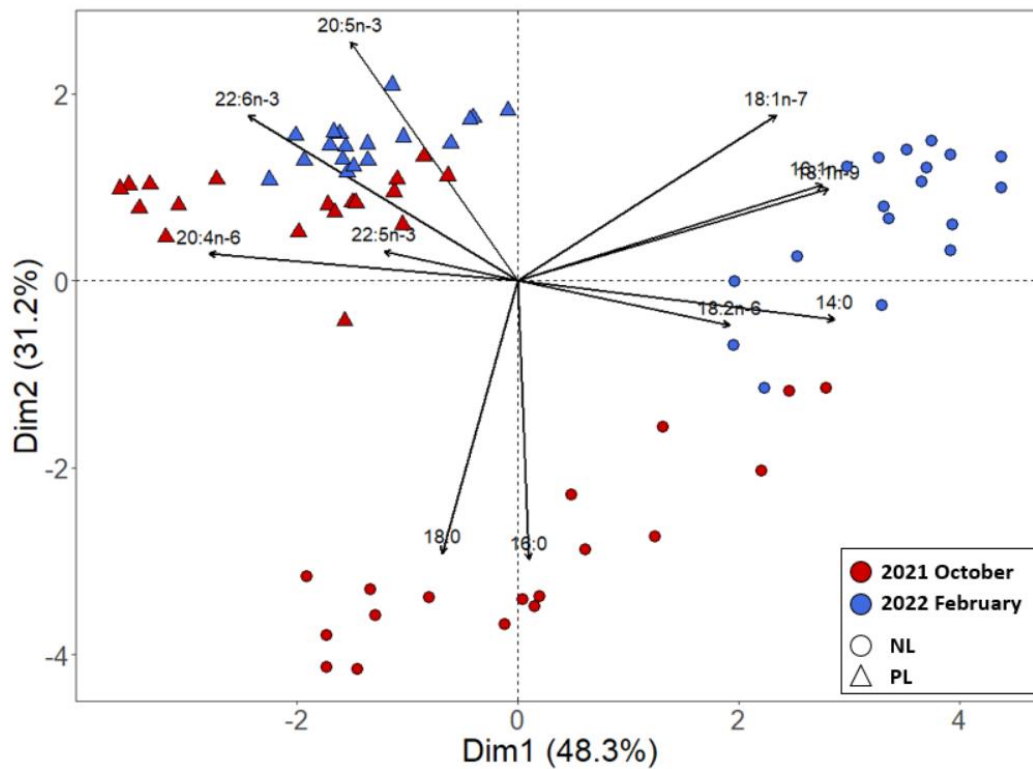


Figure 49: Principal Component Analysis of the PL and NL FA proportions in 2021 October and 2022 February.

5. Discussion

The main objectives of this study were to determine the influence of a dietary change on the FA composition in fish white muscle and to evaluate the feasibility of using a biopsy to monitor these changes. We also wanted to ensure that the biopsy did not adversely affect fish growth. We showed that it is possible to monitor the FA composition of European sea bass submitted to a dietary change using muscle biopsy.

The recorded positive growth rate throughout the study indicates that the experimental diet sustained a fairly good growth. Despite not having fish that were not biopsied to control for the effect of the biopsy on growth, no individual died or had negative growth. A previous study reported that a red muscle biopsy on *D. labrax* did not lead to any negative effect on growth nor on the immune response (Quéméneur et al., 2022). The lower condition factor measured in October, associated with its positive correlation with the percentage of NL, suggests that fish used more reserves in October than in February. As in October fish were recently caught from the wild (August), the stress of the capture, transport and acclimation can explain this

result. As the CF increased significantly from October measured in February it suggests that fish showed resilience and managed to adjust to experimental conditions, translating into higher CF and higher percentage of reserve lipids in the muscle. This also suggests that NL can serve as an indicator of fish welfare in the same way as CF.

Utilizing muscle biopsy, our study validated the shifts in FA composition in NL and PL following a dietary modification as it was shown before using traditional lethal sampling (Turchini et al., 2009).

The relative stability in PL in terms of both quantity and proportions between the two periods suggests distinct metabolism, utilization and regulation of these FA in membrane structures. Such differences between NL and PL profiles are not surprising, given that PL are essential constituents of cell membranes and may be less influenced by rapid dietary changes than storage lipids (Sargent et al., 2003).

Interestingly some FA showed consequent differences between October and February, while being in relatively similar proportions in the two experimental and commercial diets. Particularly, MUFA proportions in NL were more than two times higher in February compared to October, while the experimental diet contained less MUFA. This result can be explained by the fact that the fish in October had a low NL percentage compared to fish from February. As MUFA are particularly used as a reserve of energy, fish in October probably used their MUFA and recovered them after a longer acclimation. This suggests that wild fish are able to thrive and adjust when brought to experimental conditions. The FA composition of fish in October might also have been influenced by their diet in the wild or the transition diet. Indeed, fish from October were fed only 36 full days (and 30 of transition) with the commercial diet while fish from February were fed 130 days with the experimental diet.

6. Conclusion

Given the evident changes in FA composition following dietary transition and the consistent growth and health of fish after two biopsies, it can be inferred that white muscle biopsy is a viable technique for monitoring FA changes in fish. This minimally invasive approach offers a sustainable method to track nutritional shifts without significant impact on fish well-being. The utilization of a white muscle biopsy as a tool for tracking these changes offers a promising avenue for future research in fish nutrition and physiology.

Supplementary Materials – Chapter 4

Table S7: Fatty Acid composition (% of total FA) of neutral lipids (NL) for both biopsies. Statistics represent the p-value obtained after testing the differences between each fatty acid (one-way Anova or Kruskal-Wallis test).

NL FA	2021 October	2022 February	Statistics
14:0	1.6 ± 0.6	2.8 ± 0.6	< 0.001
16:0	26.8 ± 2.1	20.5 ± 1.9	< 0.001
18:0	17.5 ± 5.1	5.6 ± 2.6	< 0.001
∑ SFA	48.0 ± 6.0	30.3 ± 4.5	< 0.001
16 :1n-7	2.0 ± 1.7	6.8 ± 1.7	< 0.001
18 :1n-7	1.6 ± 0.5	3.4 ± 0.4	< 0.001
18 :1n-9	13.0 ± 7.0	27.6 ± 4.2	< 0.001
∑ MUFA	19.3 ± 9.1	43.2 ± 4.7	< 0.001
18 :2n-6	3.5 ± 1.4	3.6 ± 1.2	0.82
20 :4n-6	2.2 ± 1.0	0.5 ± 0.1	< 0.001
20 :5n-3	2.7 ± 0.6	6.6 ± 1.3	< 0.001
22 :5n-3	1.8 ± 0.6	1.7 ± 0.4	0.39
22 :6n-3	4.3 ± 2.8	4.4 ± 0.6	0.91
∑ PUFA	29.2 ± 4.1	24.9 ± 2.4	< 0.001

Table S8: Fatty Acid composition (% of total FA) of polar lipids (PL) for both biopsies. Statistics represent the p-value obtained after testing the differences between each fatty acid (one-way Anova or Kruskal-Wallis test).

PL FA	2021 October	2022 February	Statistics
14:0	0.5 ± 0.2	0.7 ± 0.2	< 0.001
16:0	20.0 ± 1.2	18.9 ± 0.8	0.002
18:0	7.1 ± 1.5	6.0 ± 0.8	0.008
∑ SFA	28.7 ± 2.5	26.2 ± 1.2	< 0.001
16 :1n-7	1.6 ± 0.5	2.9 ± 1.1	< 0.001
18 :1n-7	2.0 ± 0.3	2.3 ± 0.2	0.005
18 :1n-9	11.1 ± 2.8	14.4 ± 2.3	< 0.001
∑ MUFA	16.8 ± 3.7	22.2 ± 3.9	< 0.001
20 :4n-6	4.3 ± 1.3	3.0 ± 1.4	< 0.001
20 :5n-3	12.0 ± 1.0	14.7 ± 1.1	< 0.001
22 :5n-3	1.8 ± 0.3	2.3 ± 0.3	< 0.001
22 :6n-3	27.0 ± 5.0	22.5 ± 2.7	< 0.001
∑ PUFA	52.6 ± 3.6	49.9 ± 3.4	0.03

Résumé du Chapitre 4 en français

Le chapitre 4 visait à mettre en place une méthode non létale pour suivre la composition en acides gras du muscle de poissons juvéniles. Pour ce faire, une première partie a visé à développer une méthode. Nous avons utilisé six poissons sur lesquels nous avons réalisé 6 biopsies chacun ainsi qu'un prélèvement de muscle global, incluant la zone biopsiée. Nous avons testé deux méthodes d'extraction en triplicat. Une méthode Fresh qui consistait à plonger le tissu directement dans le solvant et une méthode qui consistait à lyophiliser le tissu avant. Ces méthodes ont été comparées avec une méthode de routine. Premièrement, la biopsie a permis l'obtention de chromatogrammes de qualité où l'identification des AG était simple. Les résultats ont montré qu'en termes de quantité d'acides gras totaux extraits, une grande variabilité entre et inter méthode était présente. Cette variabilité a été mise sur le compte de gouttelettes lipidiques qui auraient pu être présentes dans le tissu. En termes de quantités, les résultats se sont montrés plus convaincants. En effet, peu de différences étaient présentes entre la biopsie et le contrôle. Cela nous a permis de conclure que la biopsie était une méthode efficace pour étudier les proportions d'AG. Dans la deuxième partie de ce chapitre, nous avons voulu valider la méthode de biopsie en la mettant en application. Pour ce faire, nous avons biopsié les poissons du Chapitre 2 et 3 à leur première disposition en conditions expérimentales (Octobre 2021) et une deuxième fois au changement de Diet 1 à Diet 2 (Février 2022). Les profils en AG des lipides neutres (LN) et lipides polaires (LP) ont été comparés entre les deux dates. Nous avons également tenté d'établir un lien entre données de croissance et profils d'AG. La biopsie n'a pas entraîné de croissance négative chez les individus. Les résultats ont montré un lien entre le pourcentage de LN et l'indice de condition. Cela suggère un lien entre la santé générale d'un animal et ses taux de réserves. Les profils de LN reflétaient ceux de l'aliment de façon plus prononcée que les LP. Cependant les deux fractions suivaient la composition de la diète. Le profil général des AG était différent entre les deux biopsies. Pour la première fois, la composition en AG a pu être suivie grâce à la biopsie. Cela permet de valider cette méthode qui pourra permettre de suivre la composition en AG dans des études sur le poisson juvénile.

General Discussion

1. How is the FA composition of sea bass modulated in a natural environment?

1.1. Is the FA composition different between tissues?

The general results of this thesis highlighted a tissue specific distribution of FA (Chapter 1 & 2). In particular, we showed that the membrane lipids of liver and muscle had a LC PUFA composition that followed the same dynamics (**Figure 50**). The brain on the other hand, distinguished itself by a higher regulation of its membrane FA. Indeed, the FA composition of the brains of fish that had spent more than a year in the laboratory was relatively close to that of fish from the in-situ study (**Figure 50**). This result, although expected, confirms the brain's ability to maintain stable levels of essential fatty acids such as DHA, which makes it less vulnerable to nutritional changes compared to the liver and muscle. However, the nutritional stress induced by the severe DHA limitation for 5 months reduced the proportions of DHA in the brain (Chapter 2, **Figure 50**). It is interesting to note that, compared with the proportions of EPA in the two experimental diets (Diet 2), EPA remains in a very low proportion in the brain (**Figure 50**). This may suggest that (i) it is in sufficient quantity in the diet, or (ii) that it is not specifically retained in the brain, or (iii) that it is catabolized or used for synthesis (Chen and Bazinet 2015; Závorka et al. 2021).

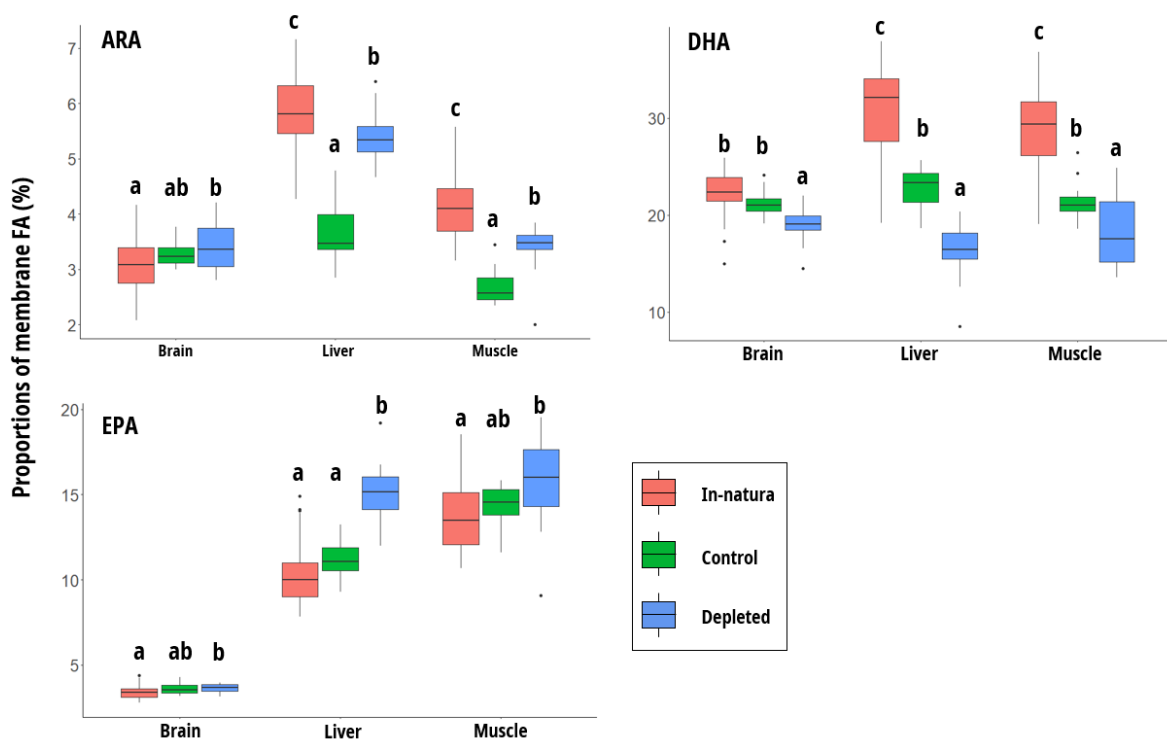


Figure 50: Distribution of ARA, DHA and EPA in the membrane lipids of the brain, liver and muscle of the different groups analysed in the thesis (In-natura: from fish sampled in their natural environment in 2019 for Chapter 1, all estuaries and ontogenetic stages were mixed; Control: fish fed the control diet2 during the experimental conditioning presented in Chapter 2; Depleted: fish fed the DHA-depleted diet2 during the experimental conditioning presented in Chapter 2). Control and Depleted fish were fish initially caught in Seine estuary in 2021. Different letters indicate statistical differences between groups among one organ ($p < 0.05$, Anova or Kruskal-Wallis). Brain: In-natura $n=49$; Control $n=18$; Depleted $n=16$. Liver: In-natura $n=75$; Control $n=18$; Depleted $n=16$. Muscle: In-natura $n=50$; Control $n=18$; Depleted $n=16$.

The organ-specific distribution of fatty acids has important functional implications. For instance, the brain's capacity to buffer DHA levels might serve as a neuroprotective mechanism, safeguarding cognitive functions and neural integrity under varying nutritional conditions (Bazinet and Layé 2014). Conversely, the liver and muscle, which are more sensitive to dietary FA fluctuations, might have greater metabolic flexibility (Madeira et al. 2021).

1.2. Does ontogenetic stage influence FA composition?

Previous studies have emphasized the significance of the ontogenetic stage in the FA composition, and suggested that its influence might even be greater than that of diet (Lane et al. 2011; Maazouzi et al. 2011; Chaguaceda et al. 2020). The LC PUFA requirements for larvae and juvenile fish are known to be different (Izquierdo and Fernandez-Palacios 1997; Mejri et al. 2021), but these requirements may also vary during the juvenile stage ((Tocher, 2010). Thus, changes in the FA composition of tissues not only reflect different diets but may also be driven by differences in requirements linked to the ontogenetic stage (Chaguaceda et al. 2020). We have highlighted these potential differences in the ontogenetic requirements of certain FA (**Figure 51**). In particular, the youngest individuals (G1) are differentiated by higher apparent requirements or retention of ARA or EPA (**Figure 51**). In contrast, 18:1n-9 (OA) which is an important source of energy for fish (Tocher, 2003) was found in lower proportions in G1 but increasing with age. The lower proportions of OA are probably due to its use for growth, as observed in other juvenile fish species (Tocher 2003; Maazouzi et al. 2011; Czesny et al. 2011).

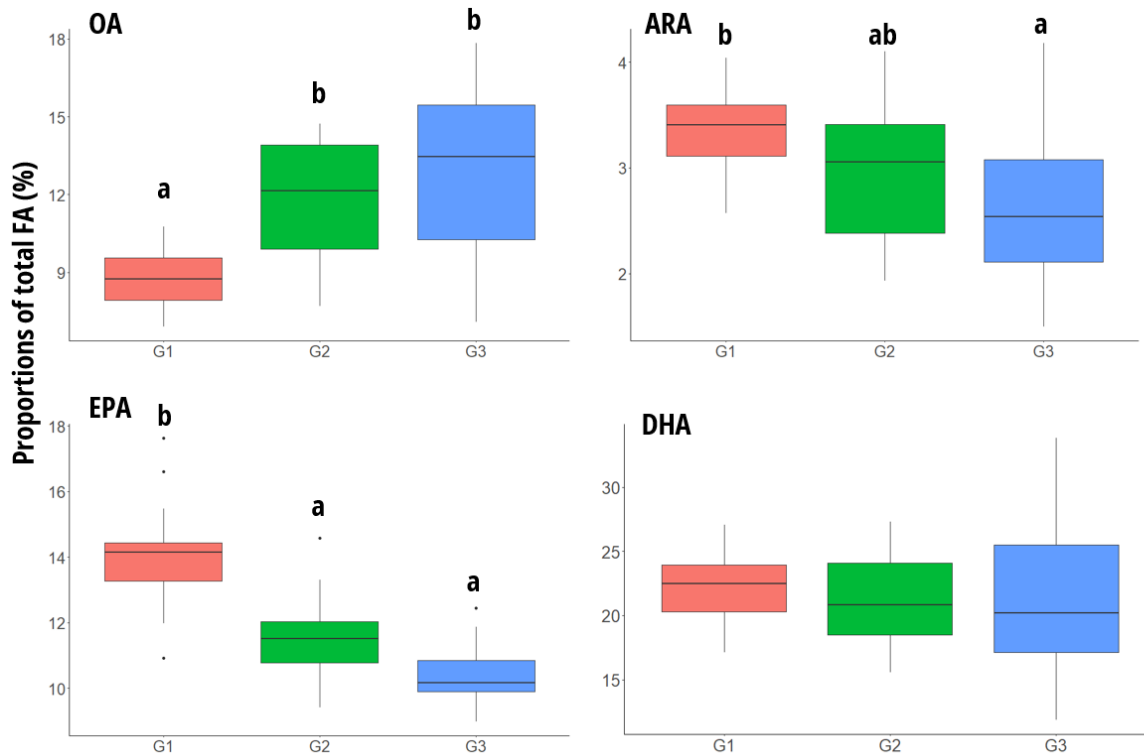


Figure 51: Distribution of OA, ARA, EPA and DHA in the total lipids of the muscle of the different ontogenetic stages (fish from Seine and Loire estuaries are mixed). Different letters indicate statistical differences between groups per organ ($p < 0.05$, Anova or Kruskal-Wallis). G1: $n=18$, G2: $n=16$, G3: $n=16$.

1.3. What is the effect of dietary FA on tissue composition

The FA composition of tissues is known to be highly influenced by nutritional (Robin et al. 2003). In both the in-natura and nutritional conditioning studies, we observed the influence of FA on tissue composition (Chapters 1, 2 and 4). The results obtained in Chapter 1 showed that even for individuals of the same age from the same origin, FA composition was highly variable between individuals. Given that European sea bass is an opportunistic predator (Pickett and Pawson 1994), the FA composition of its prey is likely to be highly variable, which translates on tissue composition. A recent study used the FA composition of wild sea bass muscle and liver to distinguish individuals by their location in the estuary (Fonseca et al. 2022). This “Fatty-Acid profiling” allowed tracking precisely the geographical origin of the fish, which can benefit the institutions that regulate illegal fishing of sea bass (Fonseca et al. 2022). Despite feeding on a diet almost devoid of DHA for more than 5 months, the fish had relatively high concentrations of DHA in their tissues. Even though the DHA proportions were lower than the Control condition, this highlight a great resilience of sea bass when put in a high nutritional

stress. These results opened the question on the mechanisms put in place by the animals to either retain or synthesize DHA in their tissue and their associated costs.

1.4. Interaction of factors and inter-individual variability

The influence of the factors on fish FA composition that have just been discussed (tissue, origin, trophic source, and ontogenetic stage) should not be considered in isolation, as there is likely an interaction between these various factors. For instance, ontogenetic stage indirectly affects food intake in the natural environment, as smaller sea bass (<20 cm) have a diet based on benthic fauna, while larger individuals (>20 cm) are predominantly piscivorous (Pérez-Ruzafa and Marcos 2014). The LC PUFA requirements that evolve through ontogenetic stages are likely different depending on the considered tissue. These interactions are crucial to take into account; failing to do so might overlook essential information. If we only consider the ontogenetic stage, G1 fish superficially show no difference in muscular DHA composition compared to G2 and G3 (Figure 51). However, we found in Chapter 1 that G1 from the Seine had a lower proportion of DHA in the muscle and liver than the other groups. This was associated with an upregulation of genes involved in LC PUFA synthesis (*fads2* and *scd1b*) suggesting an interaction between the ontogenetic stage and the estuary of origin.

The complexity of these interactions is likely a major cause of the observed variability among individuals. Interestingly, we noted that this individual variability was not necessarily higher in the natural environment, where animals are exposed to a myriad of changing parameters such as temperature, salinity, oxygen availability, or chemical stress compared to individuals reared in controlled conditions. In fact, the proportions of DHA in the muscle of fish from the Depleted condition tested experimentally (Chapter 2) were more variable than those observed in the natural environment (all ages and origins combined, $CV_{in-natura} = 13.2\%$; $CV_{Depleted} = 18.9\%$, Figure 50). In addition, most of the data obtained during this thesis concerns wild sea bass, with different life histories and different biotic and abiotic environments experienced among individuals. In contrast, domesticated farmed fish have a more homogenous genotype because of selection and, as a result, smoother phenotypes than wild individuals (Albert et al. 2012). Although the rearing conditions in experimental fish are homogeneous, these differences in life history can still influence the traits measured in reared

fish. This inter-individual variability may also be influenced by epigenetic mechanisms, which act as an additional layer of regulation beyond the genomic sequence. Such epigenetic modifications have been shown to influence the regulation of LC PUFA in multiple marine fish species, including the European sea bass (Geay et al. 2012; Turkmen et al. 2019; Perera et al. 2020; Xie et al. 2021). In addition, adjustment mechanisms linked to metabolic programming may also explain this variability. It has been shown that when juvenile sea bass are exposed to low quantities of LC n-3 PUFA at the larval stage, they over-express the genes involved in LC PUFA synthesis (Vagner et al. 2007b). In marine fish, there is a link between the proportions of DHA in the egg and the proportions of DHA at the larval and juvenile stages (Fuiman and Perez 2015; Xu et al. 2021). Similarly, broodstock can influence the proportions of LC PUFA in the tissues of their offspring. Broodstock gilthead seabream with a high expression of *fads2* induces an increase in the expression in the offspring, resulting in an increase in LC PUFA in muscle and liver (Xu et al. 2021).

2. How is the LC n-3 PUFA biosynthetic pathway modulated?

2.1. In a natural environment

Our *in-situ* study demonstrated (i) higher *fads2* expression in individuals with the lowest DHA proportions in muscle and liver, and (ii) a negative correlation between liver DHA proportion and *fads2* expression, suggesting a capacity to activate LC PUFA synthesis pathways when subjected to lower quantities in the trophic environment. Regulation of the LC PUFA synthesis pathway and associated genes is known to be complex and influenced by many factors in addition to dietary intake (Vagner and Santigosa 2011). The environment in which the fish evolve may have had an influence on the ability of individuals to upregulate these genes. Despite the higher gene expression of *fads2* and *scd1b*, the G1 from Seine did not compensate for their lower DHA proportions. This has previously been observed in nutritional studies on the same species but this is the first time that it is observed in a natural environment. Marine environment is considered rich in LC n-3 PUFA compared to freshwater systems. This dichotomy is thought to be at the origin of the differences in synthesis capacity between freshwater and marine fish (Tocher, 2010). However, estuaries are highly variable environments where the availability of LC n-3 PUFA is likely to vary according to the strata of the estuary and the seasons (Galois et al. 1996). Thus, euryhaline fish such as the European sea bass could be confronted with trophic decreases in LC n-3 PUFA, as suggested by the results of Chapter 1. This finding raises the question of the fish's ability to adjust to this change and the possible cost of this adjustment.

2.2. Facing a DHA deficiency

A modulation of the biosynthesis pathway at a molecular level was also observed in the experimental study. Fish from the Depleted condition showed overexpression of *fads2* compared to Control fish. This upregulation of *fads2* has already been observed in European sea bass fed with little or no LC n-3 PUFA in their diet (Geay et al., 2010; Vagner et al., 2007a). However, depleted fish still showed a lower DHA content than control fish. Previous studies on the same species have shown that upregulation of *fads2* does not necessarily correlate with higher activity of the associated enzyme (Geay et al., 2010). These results, coupled with

those of the in-situ study, suggest that individuals subjected to a deficiency are able to activate their synthesis pathways at the molecular level, but that this is not enough to compensate for the deficiency.

3. What are the consequences of a dietary DHA limitation?

3.1. On growth

Growth is one of the most important parameters for the survival of individuals in a natural environment (Goodrich and Clark 2023). It is particularly true in fish as they display a high growth plasticity and often face a high mortality during the first months of their life (Houde 1989; Johnston 2006). The effects of LC n-3 PUFA on fish growth have been extensively studied on numerous species in an aquaculture production context (Caballero et al., 2002; Regost et al., 2003; Tocher, 2015, 2010). In Chapter 2, the growth of Depleted individuals was negatively impacted by the 5 months of dietary DHA limitation compared to Control individuals. Slower growth was expected in the Depleted individuals as DHA proportions were very low (0.5% TFA), although the total sum of EPA+DHA in the diet was above (1.8% DM) the known requirements for juvenile European sea bass (0.7% DM; Skalli and Robin, 2004). A negative relationship was observed between the proportion of DHA in muscle and TGC (Chapter 2), as previously observed in other species (Murray et al. 2014; Závorka et al. 2021). This suggests an energetic cost for maintaining high proportions of DHA in tissues.

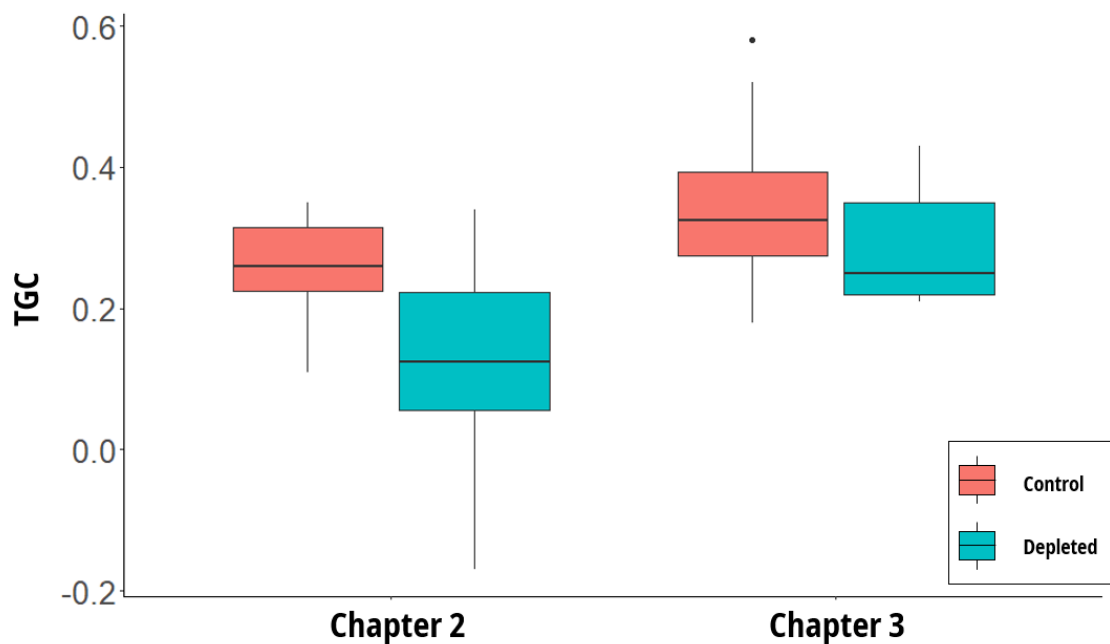


Figure 52: Thermal Growth Coefficient for both dietary conditions of fish used for Chapter 2 and for Chapter 3, and fed the Diets2. The TGC was calculated from the first day of the experiment (21 Feb. 2022). The final weight of chapter 2 corresponds to the day of their sacrifice after the swimming challenge. The final weight for chapter 3 corresponds to the weight measured on 01 Sep. 2022 when they were fed with Diet 2. Chapter 2: Control n= 15; Depleted n=16. Chapter 3: Control n=12, Depleted n=9.

However, considering fish used for Chapter 3 and fed with the Diet2 (211 days of conditioning in total), the TGC was not impaired anymore (**Figure 52**). This contrary result can be explained by a bias in the experimental design for chapter 3. Indeed, the smallest individuals were selected for the swimming tunnel challenge because of the size of the swim chamber that could not fit individuals that were more than 25 cm long. This led to an indirect selection of larger individuals used for the Chapter 3, potentially with higher growth rates (**Figure 53**) and faster growth phenotypes than those in Chapter 2. Growth phenotypes in fish can be separated into fast-growing and slow-growing phenotypes (Biro and Post 2008). Wild fish typically exhibit greater genetic and phenotypic diversity than farmed fish, which are bred for rapid growth. It is possible that they displayed high inter-individual variability in terms of growth, especially in fish fed a DHA depleted diet in Chapter 2 (**Figure 52**). This variability possibly reflects variation in the response to DHA limitation. Indeed, this high variability between individuals in the Depleted condition is found both in terms of growth (**Figure 52**) and in terms of the proportions of DHA in the tissues (**Figure 50**).

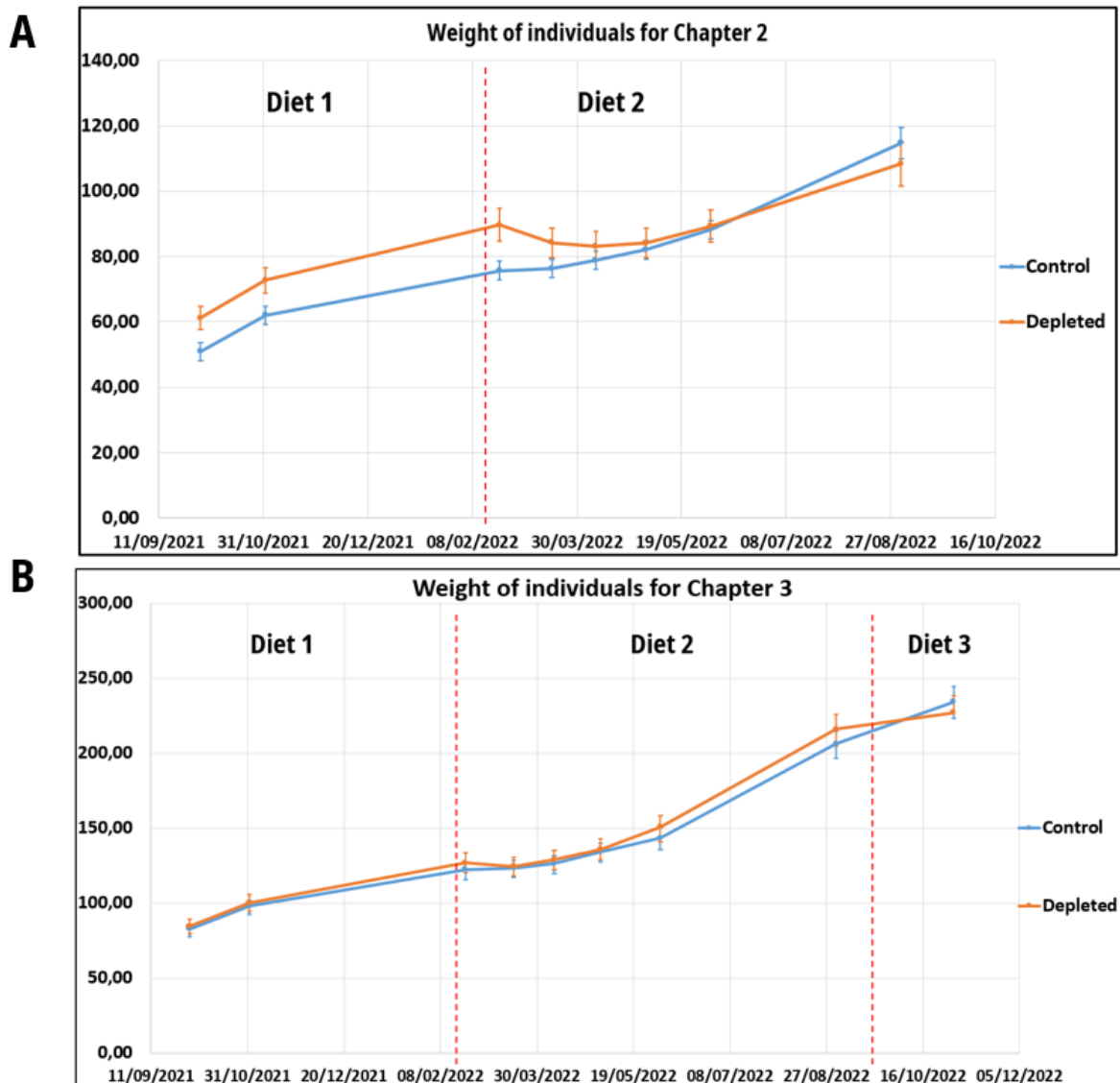


Figure 53: Growth curve for the duration of the experiment for Chapter 2 (n= 31) (A) and chapter 3 (n=21) (B). The red dotted lines correspond to a shift of Diet (e.g from Diet 1 to Diet 2).

3.2. On physiological and behavioral performances

Although DHA deficiency had a negative effect on the growth of some individuals (Chapter 2), it had no impact on metabolic or swimming performances (Chapter 2) or the ability to escape from a predator (Chapter 3). The parameters measured in Chapter 2 (Metabolic rate and swimming performance) did not show any link with the FA composition of the organs studied (liver, muscle, brain). Few studies reported a relationship between FA and metabolic rate and/or swimming performance (McKenzie et al. 1998; McKenzie 2001; Vagner et al. 2014).

Given that the Depleted fish had lower proportions of DHA in all tissues (muscle, liver, brain), it is possible that there are complex relationships between membrane composition and metabolic function. This also suggests a certain resilience of wild individuals to nutritional DHA limitation, which seems to enable them to cope with this stress at a metabolic level. This resilience was also observed in the behavioural response. No effect of nutritional conditioning was observed on escape response parameters when faced with a predator (Chapter 3). A hypothesis would be that EPA, present in high quantities in both diets, would be sufficient to maintain metabolic and behavioural performances.

These results can be explained by the change in diet 30-45 days before the experiment. Although this diet was deficient in DHA compared to the nutritional requirements of sea bass, it contained five times more DHA than the previous diet. Rapid incorporation of DHA could therefore have taken place thus ensuring maintenance of behavioural abilities. Given that the FA data for Chapter 3 are not yet available, it is difficult to speculate on whether or not the level of tissues, and particularly the brain, essential in the behavioural response are similar to those of Control fish. An additional reason for this non-significance would be the high variability between individuals in the measured variables. Wild fish are known to have greater behavioural variability among individuals than their domesticated counterparts do (Benhaïm et al. 2012). Data obtained in the wild suggest that some individuals may be deficient in DHA in the brain tissue. Indeed, the lowest levels of DHA found in the brains of Chapter 1 fish (Loire G2) are similar to those of fish fed Depleted Diet 2 for 5 months ([Figure 54](#)). Considering the crucial importance of DHA for behavioural response, this might have implications for these fish in the wild. However, as Depleted fish did not show any differences in the ability to escape a predator compared to Control, this might also indicate that these levels of DHA, though they are lower, are not detrimental for behavior.

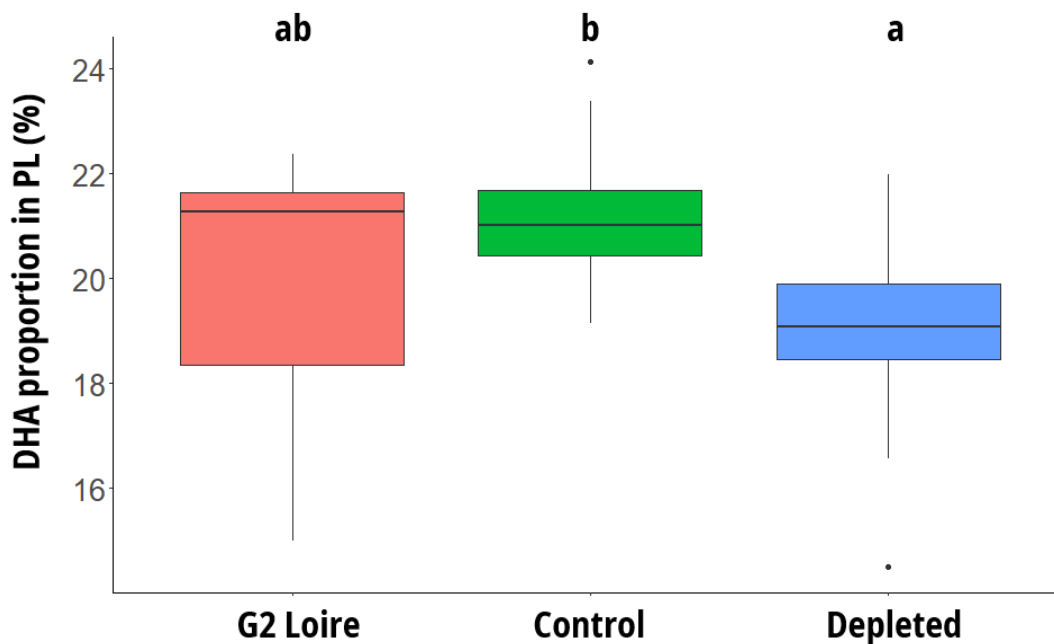


Figure 54: DHA proportion of membrane lipids (PL) in the brain of G2 Loire (n=8), Control (n=15) and Depleted (n=16) fish from chapter 2 (fed the Diets2). Different letters indicate significant differences between the groups ($p < 0.05$, one-way Anova).

4. A new method to study fatty acids on juvenile fish

4.1. The muscle biopsy: a promising method

In addition to the work carried out in situ and under experimental conditions during this thesis, a method for analyzing FA using white muscle biopsies has been developed (Chapter 4). The development of this method (Chapter 4, part 1) and its application (Chapter 4, part 2) has enabled us to prove its effectiveness in monitoring the FA composition of juvenile fish without inducing mortality. The results on fish FA composition from the natural environment (Chapter 1) or under experimental conditions (Chapter 2) have shown that the modulation of FA at the individual level is complex and likely to vary from one individual to another. The biopsy method could be used in both contexts to increase our knowledge of the modulation of FA composition. For example, the use of biopsy in parallel with capture-mark-recapture studies could provide information on the modification of a fish's FA without having to sacrifice the individual. Regular monitoring would provide information on the seasonal dynamics of FA composition, and on the turnover of FA in muscle tissue. This biopsy method has also been

previously used to monitor mitochondrial performance in red muscle of sea bass juvenile (Quéménéur et al. 2022), and to analyse heavy metal contamination or stable isotopes in other fish species (Peterson et al. 2004; Cano-Rocabayera et al. 2015). These non-destructive methods should continue to be developed to continue to bring knowledge while maximizing animal welfare.

4.2. A tool to better understand interindividual variability

The biopsy method also allowed us to better monitor inter-individual variability in muscle FA composition and observe how it changes over time. This monitoring revealed that the proportions of DHA do not evolve in the same way in individuals fed on the same diet and reared under the same rearing conditions (Figure 55). Similarly, this provides us with information on the evolution of variability between individuals over the course of the experiment. Here, conditioning from 2022 February to 2022 August with Control Diet 2 considerably reduced the variability in individual DHA proportions (Figure 55).

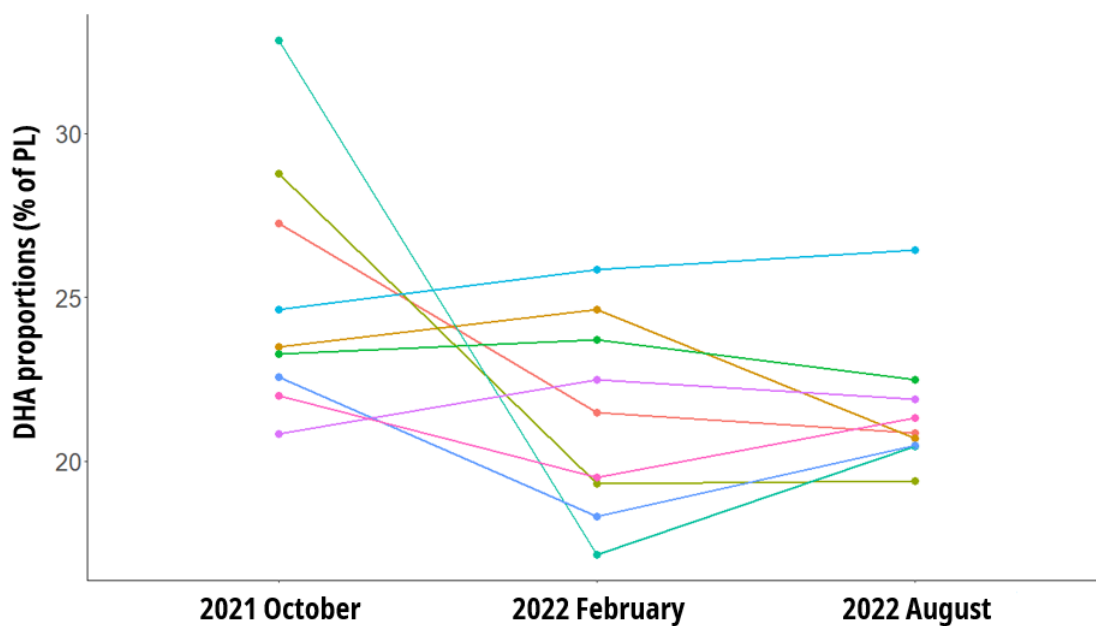


Figure 55: Individual trajectory of DHA proportion over time (See Chapter 4; n= 9 individuals). Each line represents one individual. In 2021 October, individuals were exiting quarantine after being fed 30 days of a mix of frozen seafood and commercial pellets + 36 days of commercial pellets only. From 2021 October to 2022 February, they were fed Control Diet 1. From 2022 February to 2022 August, they were fed Control Diet 2.

5. *An innovative methodological approach*

5.1. Using wild fish in experimental studies

To select a more ecologically relevant model, fish caught in the wild were used in this thesis. In theory, this approach takes experimental approaches one-step further. However, it is essential to discuss the strengths and limitations of using such models. Wild fish are likely to have a different genotype and increased phenotypic variability from fish from domesticated lines bred for aquaculture production (Albert et al. 2012). The latter are often selected for superior growth traits, which could bias the data obtained from these individuals (Millot et al. 2010). Additionally, domesticated fish tend to have a higher tolerance to stress caused by human interactions (Ashley 2007). In experimental settings, this stress can be quite pronounced due to necessary maintenance activities, such as cleaning tanks, measuring physico-chemical parameters, or conducting repeated measurements to monitor growth. All these factors undoubtedly stress the fish, and this stress is probably much higher in fish that lived in the wild before being brought to the lab (personal observation). Moreover, the logistics of using wild fish under experimental conditions can be challenging. During this thesis, transporting the fish caught from the Seine in August 2021 took about 7 hours, during which the fish experienced high stress from the trawl capture and transport. Trawl capture is a source of physical stress and the fish showed minor injury marks related to it (personal observation). Though the mortality rate upon arrival at the lab was relatively low (~30%), there was an indirect selection of fish that survived that could potentially bias the results. The need to quarantine the fish and continually monitor for potential diseases are considerations to take into account when designing experiments using wild fish. Additionally, due to the limited number of fish brought to the lab (n=101), preliminary tests in swim-tunnel respirometers were performed with farmed fish, which did not show the same stress levels as the wild fish further did (personal observation). Preliminary tests for escape response measurements were conducted with wild fish, which displayed promising results by comfortably exploring their new surroundings. However, during the escape response test (Chapter 3), while some fish were keen on exploring their environment, others did not take this risk at all (personal observation). This explains the significant disparity between the

number of videos obtained from the tested fish (n=21) and the number of fish that should have been tested but were not because they were not properly positioned in the tank (n=36). European sea bass is a gregarious species during its juvenile stage, and a hierarchy is being established within each tank. Some fish exhibit a "Shy" phenotype, while others are "Bold", depending on their "personality" (Sneddon 2003; Millot et al. 2009). A potential solution for future studies would be to test fish in groups of multiple individuals to minimize stress effects (Carvalho et al. 2022). Using enrichments inside the acclimation tanks could also be a way to mimic the natural environment and improve the relevance of behavioral studies on fish.

5.2. Coupling in-situ and experimental

This thesis tried to combine two complementary approaches with the in-situ study that brought information on the actual situation of the sea bass and the experimental study that tried to assess the effects of a predicted reduction in LC n-3 PUFA. Considering the global decline in DHA production, the findings from this thesis provide insights into an individual's ability to cope with a deficiency in the natural environment. The low proportion of DHA in Seine G1 suggests that, in the natural environment, some individuals experience dietary limitations in specific PUFA (**Figure 56**). This could be associated with increased fatty acid requirements during certain phases of the juvenile period. Low levels of DHA coupled with a predicted reduced DHA production might have detrimental effects on these individuals in the wild.

However, we showed experimentally in Chapter 2 that despite having reduced DHA content in tissue, Depleted fish managed to maintain their aerobic and swimming performance (Chapter 2) and their behavioural capacities were not affected (Chapter 3). These data suggest that DHA limitation might not have a direct influence on these parameters. However, the relationship between dietary deficiency and growth rate needs further clarification, as this was not observed in Chapter 3. Energy trade-offs related to synthesis or selective retention might be adopted by deficient individuals to maintain their tissue DHA levels (Chaguaceda et al. 2020; Závorka et al. 2021), which could influence other parameters.

It is important to note that the fatty acid amounts and profile in the experimental diets did not necessarily reflect those of the fish's prey in nature. In fact, European bass is an opportunistic predator feeding on various prey, depending on its age, and each prey has its

unique fatty acid composition that may vary seasonally (Pickett and Pawson 1994; Pérez-Ruzafa and Marcos 2014). All of this being reflected in the individual FA profiles (Chapter 1). Younger individuals presumably have the least DHA in their diet as they feed on prey with low DHA levels (crustaceans), yet DHA is most crucial for them (Dabrowski 1986).

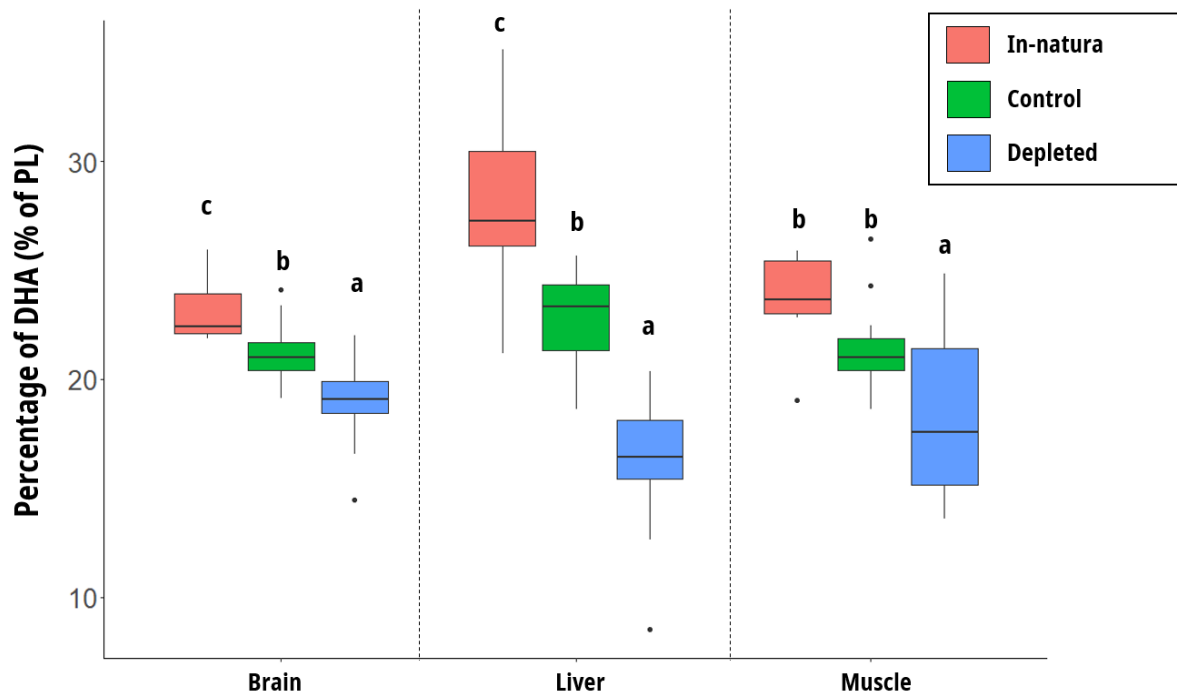


Figure 56: DHA proportions in polar lipids (PL) of brain, liver and muscle of Seine G1 (caught in 2019) from the natural environment and Seine G1 (caught in 2021) that faced a 5-month nutritional conditioning (Depleted diets 2).

6. Perspectives

The results obtained throughout this thesis provided important information on the relation between LC PUFA and the biology of the sea bass. However, it opened new research questions that deserves to be further investigated.

6.1. How effective is the synthesis of LC PUFA?

As we showed that upregulation of genes involved in LC PUFA, biosynthesis was not enough to compensate for the levels of LC PUFA in the tissues; one may wonder what is the real efficiency of the biosynthesis in the sea bass. Previous work have been using isotopes to study the biosynthesis of LC PUFA but all these studies have been conducted in domesticated fish

(Mourente et al., 2005; Mourente and Dick, 2002). Further investigations on the activity of the enzymes involved in this synthesis and the efficiency of this synthesis could shed light on whether or not the fish synthesize these molecules.

6.2. Is EPA enough to maintain metabolic and behavioral functions?

We posited that the abundant presence of EPA in both the diet and the tissues might play a pivotal role in sustaining metabolic and behavioral functions (Chapters 2 & 3). To provide a more rigorous evaluation of this premise, a future avenue of investigation should examine the repercussions of dietary EPA depletion. Given the distinct metabolic roles played by EPA and DHA, such a study could bring more nuanced insights into the specific requirements and implications of EPA in the physiology of sea bass, thereby augmenting our comprehensive understanding of its dietary significance.

6.3. Pushing the in-situ further

Combining the in-situ and experimental study in a pivotal point of this thesis. It allowed for comparison between the actual situation and a predicted one to better understand the effects of climate change induced nutritional stress. To push this comparison further, we could investigate the metabolism of fish in a natural environment. Using field respirometers, it is possible to monitor the oxygen consumption of freshly caught individuals in the wild. Considering the stressful character of this experiment, this might only provide us with information on the maximum metabolic rate of these individuals but it might offer a point of comparison with data obtained in a laboratory experiment.

6.4. What are the effects of DHA depletion on other parameters?

6.4.1. Immunity and health

Certain parameters could be worthy of study in a context of DHA deficiency. Indeed, the benefits of this FA are numerous and certain parameters not measured during this thesis could be impacted by a nutritional deficiency. LC n-3 PUFAs are known to have effects on organism immunity (Kumar et al. 2022). Although EPA plays a predominant role in the immune response because it is a precursor of eicosanoids (Rowley et al. 1995), DHA also has an anti-

inflammatory role (Calder 2009). Studying the health of fish subjected to a deficiency could be of interest in order to increase our knowledge of European sea bass in an aquaculture context.

6.4.2. Reproduction

LC n-3 PUFA including DHA plays a role in reproduction and particularly when the fish is maturing (Izquierdo et al. 2001). At the end of the experiment, some individuals reached size that are close to that of mature sea bass (Pickett and Pawson 1994). It would be interesting to study the effects of the long-term depletion on the reproductive organs of wild sea bass as, to our knowledge; this would be the first study of the kind. Samples of reproductive organs were taken at the end of the experiment (1 year and 3 months after fish arrival to the lab) to carry further FA composition, molecular and histological analysis.

6.4.3. Mitochondrial function

Considering our analyses on the metabolic performances and growth, mitochondrial performance would be another trait worth studying in link with DHA depletion. Mitochondrial is the powerhouse of cells and produce ATP fuelling most physiological processes (Vakifahmetoglu-Norberg et al. 2017). Recent studies linked the mitochondrial performance with other more integrative traits such as growth and overall animal performance (Salin et al. 2015, 2019). Mitochondrial membrane can be modified by dietary changes in LC n-3 PUFA which in turn can have repercussion on organism performances (Závorka et al. 2021; Salin et al. 2021). Studying the link between mitochondria and dietary DHA depletion could thus be of interest to try to establish links between various traits of performances.

7. *General conclusion*

In conclusion, this thesis has expanded our understanding of FA composition and its modulation in wild fish under natural conditions and when subjected to DHA deficiency. Using wild fish as a model brings more authenticity to the study as it represents a closer approximation to natural ecophysiological conditions. The in situ analysis not only supports prior findings on the ontogenetic and spatial modulation of FA in tissues but also underscores the intricate interactions between these determinants.

We observed that DHA deficiency can influence growth in wild fish, yet these individuals manage to sustain their metabolic performance during swim tests. Drawing definitive conclusions regarding the effects of DHA deficiency on behavioral performance, especially in predator evasion situations, remains complex. Nonetheless, these insights pave the way for further research to delineate the nuanced impacts of DHA deficiency on survival and fitness outcomes in wild fish populations.

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Appendix

1. Formulation of the diets

		Aliment standard	Aliment appauvri en DHA
Farine de Poisson		20	20
Protéines %	74,47	14,136	14,136
Lipides %	5,89	1,118	1,118
EPA+DHA % lip	20,4	0,216	0,216
EPA %	7,2	0,076	0,076
DHA %	13,2	0,140	0,140
ARA %	2,4	0,025	0,025
MS %	94,91		
% des lipides			
Gluten de blé		50	50
Protéines %	83,99	38,959	38,959
Lipides %	1,97	0,914	0,914
EPA+DHA % lip	0	0,000	0,000
MS %	92,77		
	lipides %	2,032	2,032
	EPA+DHA	0,216	0,216
Huile de colza		3	5
EPA+DHA % lip	0		
lipides		100	100
EPA %		0	0
DHA %		0	0
ARA %		0	
Huile de poisson		7	0
EPA+DHA % lip	25	1,750	
Lipides		100,000	
DHA %	15	1,050	
EPA %	10	0,700	
ARA %	0,8	0,056	
Huile polaris micro algue		0	5
EPA mg/g	294		
EPA %	32,97		1,6485
DHA %	0,03		0,0015
ARA %	3,79		0,1895
Amidon		6	6
Vitamines		4	4
Minéraux		2	2
Cellulose		5	5
Bétaïne		3	3

Figure S7: Formulation of the experimental diet

2. Quantities

You need to allow for 140-150% more than the desired quantity because of losses throughout the process.

Ex: For 3.5 kg of finished feed, allow around 5kg.

We have enough raw material to make 10kg of feed in case of a problem.

We decided to make the feed in batches of 2.5kg.

3. Procedure for 2.5kg

3.1. Weigh and mix the powders

To make the dough, use only the HOBART mixer on speed 1 with the flat beater. The powders must be added progressively and one by one (at each stage, manually incorporate the powder into part of the mixture before putting everything into the bowl) to the mixture in the following order:

- Sieve the fishmeal through a 500µm sieve
- Weigh out 500g of sieved fishmeal
- Weigh and add 75g Betaine
- Create the vitamin mix by including vitamin C (ascorbic acid) in the mix already existent:

Vit.C is 35%, so for 100g of mix you need to add ($C1 \times V1 = C2 \times V2$): $V1 = (43\% \times 0.68) / 35\% = 0.83g$

Vit.C

99.17g Vit. mix + 0.83g Vit.C = Vit. Mix.

- Add 100g of Vit.

Clean the spoons between each powder to avoid mixing products and/or keep one container per product.

- Weigh and add 50g of Mineral mix

Vitamin mix and Mineral mix must not be mixed directly together, as this destroys the vitamins.

- Weigh out and add 1250g of Wheat Gluten (add directly to the bowl without mixing by hand)
- Weigh and add 150g of Gelled Starch
- Weigh and add 125g Cellulose

3.2. Adding the oils

- Weigh and mix 125g rapeseed oil and 125g Polaris oil in a second bowl.
- Gradually incorporate the powder mixture into the oil.

Stop the machine regularly to remove the dust from the mixer and to homogenise the paste by hand.

3.3. Adding water

While mixing, gradually add 25-30% of the total weight in hot water (does not burn the hand).

The amount of water depends on the texture of the dough. A gluten-rich dough absorbs more water.

- Stop pouring the water when the mixture :

- clumps together into crumbs
- is no longer floury: the powder no longer sticks to your hands
- holds together in a ball but crumbles easily



If the dough does not contain enough water, it will crumble as it passes through the die. Simply mix the dough again, adding a little water. It is better not to add enough water than too much, and to add it gradually.

3.4. Chopping the dough



Figure S8: Setup of the processors

2 knives are needed : 1 with 4 handles for the inside and 1 with 5 handles for the outside.

The cutters must be positioned so that the blades stick to the die.

The bolt should be barely tightened so as not to force the mechanism

- Die of the required pellet size (here: 4 mm)
- Grinding at speed 1
- Gradually feed the powder into the system
- Be careful to check through the hole that there is no agglomeration of paste (this happens when the paste is too wet or the die is blocked).
- Clean the machine

3.5. Drying the diet

- Clean the suction columns by repeatedly pulling on the bottle
- Clean the white carpet
- Spread the dough fragments evenly in the bottom of the drying vat
- Insert the tank under the dryer and unhook it

- Push the "SEAL" lever to activate the air cushion and make the drying vat stick to the upper part.
- Start drying for 15 minutes at 50-60°C - "START" button on the right-hand side
- Stop the suction, remove the tank and mix the granules
- Dry for a further 10 minutes at 50-60°C

3.6. Cleaning

- Wash all the equipment
- Clean used dies in a muffle furnace (400°C program)

4. Remarks

Extrusion is the process that combines pressure and heat to transform the raw material (flour, oil, etc.) into a homogeneous finished product. The cooking process involves the gelatinisation of the starch. In effect, the starch is broken down and used as a binder for the finished product (creation of a matrix). If an extruder is not used, already gelled starch must be added to the raw material. By varying the extrusion parameters, it is also possible to vary the flotation behavior of the pellet. If no extruder is available, gelled starch powder can be used as a substitute.

Titre : Rôle de l'habitat actuel et du stade ontogénique sur la capacité du bar (*Dicentrarchus labrax*) à faire face au scénario futur de baisse de disponibilité en oméga-3 polyinsaturés à longue chaîne dans le réseau trophique

Mots clés : Bar européen, Acides gras, Métabolisme, Comportement, DHA, Physiologie

Résumé : Les acides gras polyinsaturés à longue chaîne (AGPI LC) et particulièrement de la famille des oméga-3 (AGPI LC n-3) dont l'acide docosahexaénoïque (DHA) et eicosapentaénoïque (EPA) sont des molécules essentielles pour le développement et le maintien des performances physiologiques et comportementales chez les organismes dont les poissons. Cette thèse a visé à comprendre la modulation de ces AGPI dans le milieu naturel et à étudier l'effet d'une carence en AGPI LC n-3 sur les performances métaboliques, de nage et comportementales du bar Européen en expérimental. Dans le milieu naturel, la composition en AGPI est modulée par l'interaction entre le stade ontogénique, l'estuaire d'origine et l'apport trophique. Certains individus font, à priori face à des niveaux bas en AGPI LC n-3 ce qui entraîne des mécanismes d'ajustement au niveau moléculaire.

Les bars européens sauvages ramenés au laboratoire ont été conditionné avec un aliment dépourvu de DHA pendant plusieurs mois. Sur une période de 5 mois, l'aliment carencé a entraîné un retard de croissance mais n'a pas impacté les performance métaboliques ou de nage des individus. Les poissons carencés ont maintenus des haut niveau de DHA dans leurs tissus montrant une résilience face à la carence. Le conditionnement nutritionnel n'a pas non plus impacté la capacité du bar Européen à échapper à un prédateur même si certaines tendances ont mis en évidence un temps de réaction augmenté chez les individus carencés. Cette thèse a contribué à l'enrichissement des connaissances sur le bar européen et sur la relation entre AGPI LC et les réponses métaboliques et comportementales sur une modèle pertinent qu'est le poisson sauvage.

Title: Role of current habitat and ontogenetic stage on the ability of sea bass (*Dicentrarchus labrax*) to cope with future scenario of declining availability of long-chain polyunsaturated omega-3s in the food web

Key words: European sea bass, Fatty acids, Metabolism, Behaviour, DHA, Physiology

Abstract: Long-chain polyunsaturated fatty acids (LC PUFAs), particularly from the omega-3 family (LC n-3 PUFA) including docosahexaenoic acid (DHA), are essential molecules for the development and maintenance of physiological and behavioural performance in organisms including fish. The aim of this thesis was to understand the modulation of these PUFAs in the natural environment and to study the effect of LC n-3 PUFA deficiency on the metabolic, swimming and behavioural performance of European sea bass in experimental conditions. In the natural environment, the PUFA composition is modulated by the interaction between the ontogenetic stage, the estuary of origin and the trophic input. Some individuals tend to have low levels of LC n-3 PUFAs, which leads to adjustment mechanisms at the

molecular level. Wild European sea bass brought back to the laboratory were conditioned on a DHA-deficient feed for several months. Over a period of 5 months, the deficient feed caused a delay in growth but had no impact on the metabolic or swimming performance of the individuals. The deficient fish maintained high levels of DHA in their tissues, showing resilience to the deficiency. Nor did nutritional conditioning have any impact on the ability of European sea bass to escape from a predator, although some trends showed an increased reaction time in deficient individuals. This thesis has contributed to our knowledge of European sea bass and the relationship between LC PUFAs and metabolic and behavioural responses in a relevant model, namely wild fish.