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RESEARCH

Non-lethal sampling method for the analysis of white muscle fatty acid profiles in European sea bass (*Dicentrarchus labrax*)

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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.

Keywords Fatty acids · European sea bass · Muscle biopsy · Lipid composition · Method comparison

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 signaling and communication (Murphy 1990) and 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, and organs that ultimately affect the overall performance of an individual (Tocher 2010). 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, and development (Tocher 2010). However, as for most animals, these FA are weakly synthetized de novo by

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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 2009) 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. 2021) 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 non-lethal 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.

Material and methods

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.

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 S1).

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) (Fig. S1). 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 h 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 (Fig. S1). MC were treated following our routine protocol for FA analyses. It was flash frozen and 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.

Analysis of the muscle fatty acid composition

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 × 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.

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.

FAME analysis

Analyses of FAME were performed on a Varian CP8400 gas chromatograph using simultaneously two separations on a polar column (DBWAX: 30 mm × 0.25 mm ID × 0.2 µm, Agilent) and an apolar column (DB5: 30 m × 0.25 mm ID × 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 and 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 (µg.mg^{-1} WW). Fatty acid proportion was defined as the mass percentage of each fatty acid to the total fatty acid content.

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 ± SEM.

Results

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 ± 13.5 µg (min–max, 5.5–409.0 µg) of FA for NL and 28.8 ± 1.7 µg (mix–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 ± 13.5 µg (min–max, 5.5–409.0 µg) of FA for NL and 28.8 ± 1.7 µg (mix–max, 10.8–45.6 µg) of FA for PL (not shown).

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 (Fig. 1 NL A) (Fresh: min–max, 1.0–98.4 mg.g^{-1}; FD, min–max, 0.7–74.4 mg.g^{-1}) this variability, illustrated by the coefficient of variation of the three replicates per individual and biopsy method (CV mean = 75 ± 40%, min–max = 15–147%) was

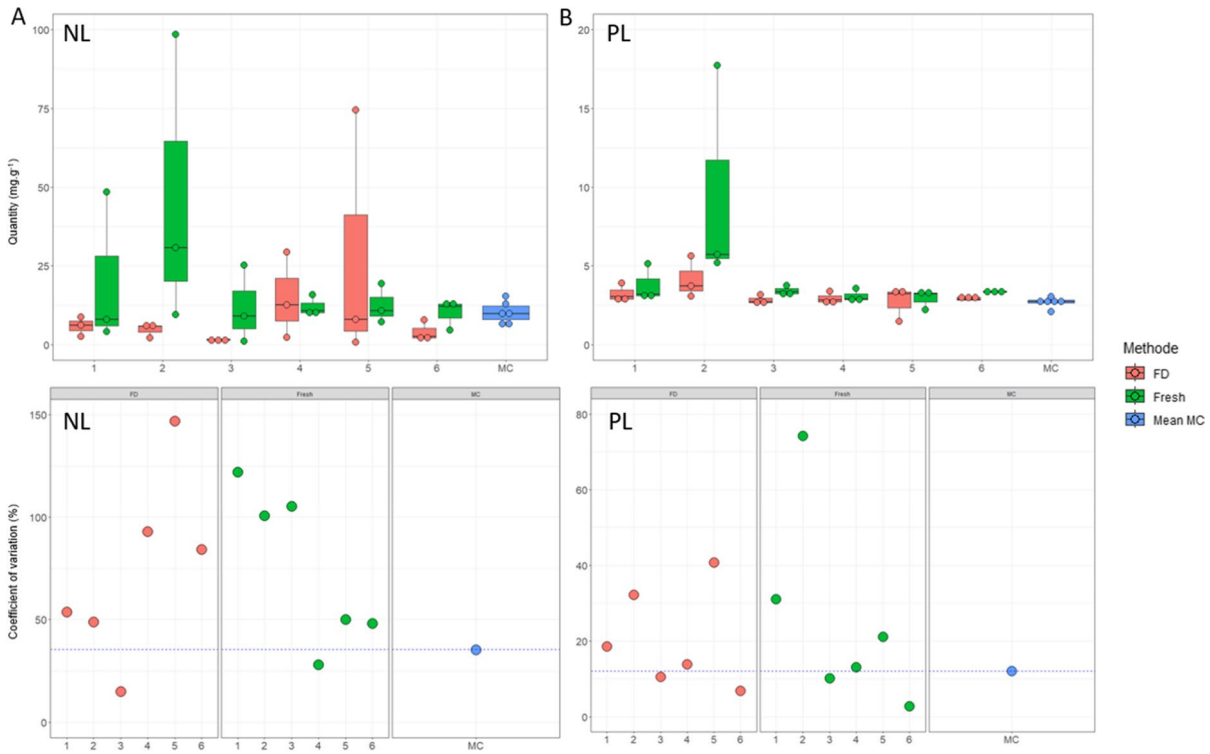

Fig. 1 Fatty acid quantities (mg.g^{-1} WW upper panels) and coefficients of variation (percentage, lower panels) of FA quantities in non-polar lipids (**A**, **C**) and polar lipids (**B**, **D**) of different method. The different methods are presented as followed: freeze-dried protocol (FD, red, $n = 3$ 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 three (for biopsies) or six replicates (for MC), and the blue dotted line represents the value of MC CV

always above 10% (Fig. 1C). The CV values were higher that those of MC for five out of six individuals for both methods. In PL, the intra-individual variability of total FA quantity was lower than in NL (Fig. 1B) but still displayed considerable differences between extreme values (Fresh: min–max, 2.2–17.7 mg.g^{-1}, FD, min–max, 1.4–5.6 mg.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 ± 19.8%, Fig. 1D). The CV values were higher than those of MC for four out of six 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 (Fig. 2A) varied less within individuals (CV <10%) than EPA and DHA (Fig. 2A, E, and F, 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 (Fig. 2B).

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 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 (Fig. 2).

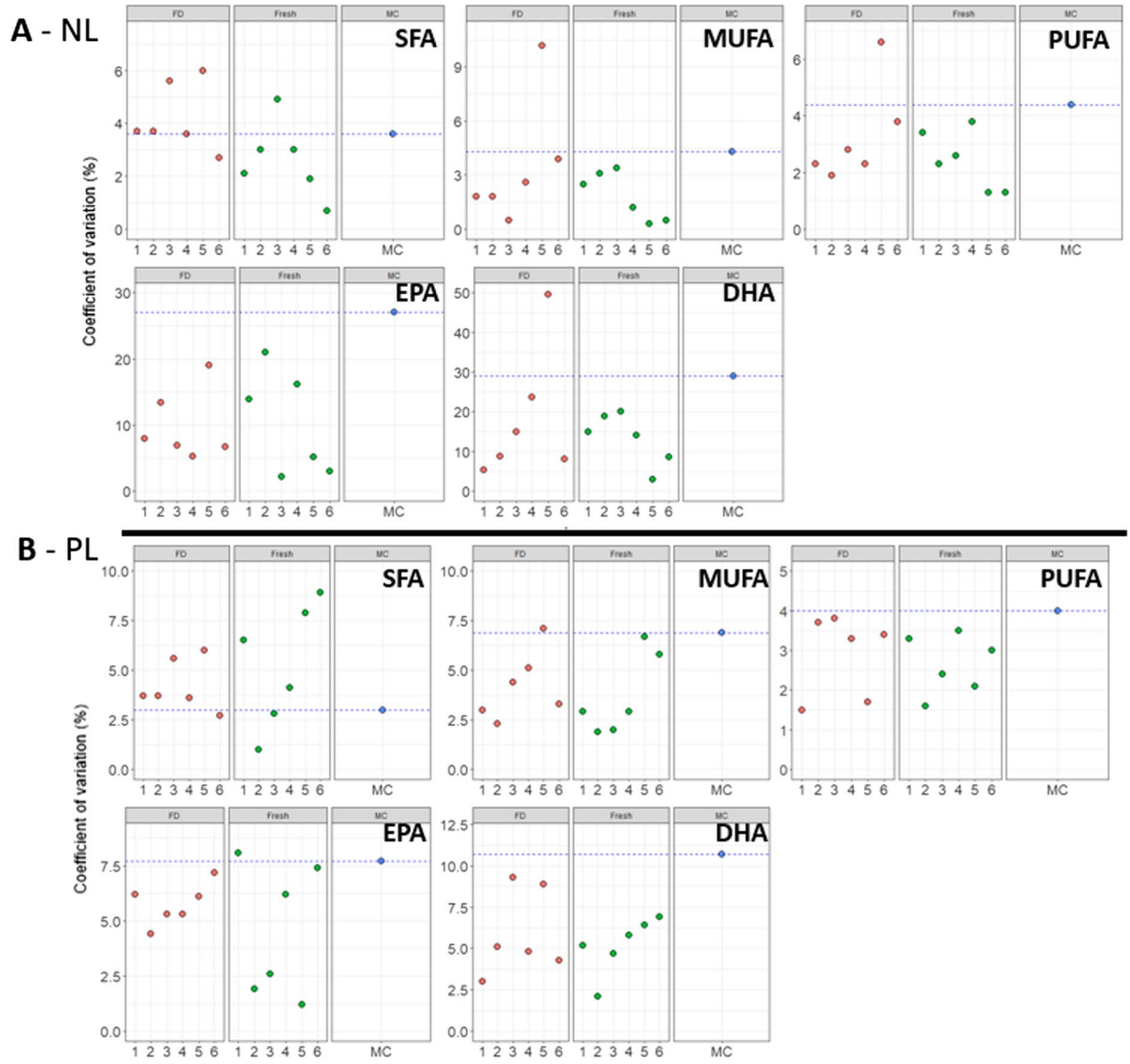

Fig. 2 *Coefficient of variation (percentage) of FA proportions in NL (***A***) and PL (***B***) for the different methods.* Main family and FA are presented: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (EPA), and docosahexaenoic 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 represents the CV of the three (for biopsies) or six replicates (for MC), and the blue dotted line represents the value of MC CV

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 ten major fatty acids (Fig. S2), 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 1), and consequently, SFA proportions followed this trend. No statistical differences were observed among the three methods for PL FA proportions (Table 1).

Table 1 Fatty acid proportion (mass percentage of each FA over total fatty acids) of the ten 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*

NL fatty acid	Muscle biopsies		Muscle control	Statistics
	Fresh	Freeze-dried		
14:0	2.9 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	-
16:0	18.1 ± 0.3[a]	18.2 ± 0.1[a]	18.2 ± 0.1[a]	**
18:0	3.7 ± 0.2[a]	3.6 ± 0.1[a]	3.6 ± 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	-

Data are presented as inter-individual means (± 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

Discussion

Adequacy of 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, and 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 76.7 g 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 (4 mm compared to our 2 mm) on Moses perch *Lutjanus russellii* 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.

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 the 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.

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.

Conclusions

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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Data Availability All data will be made available on request.

Declarations

Ethics approval 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.

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

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