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Post-mortem storage conditions and cooking methods affect long-chain omega-3 fatty acid content in Atlantic mackerel (*Scomber scombrus*)

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Abstract :

Long-chain omega-3 fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) are health beneficial lipids found in high concentration in pelagic fishes, including Atlantic mackerel. While EPA and DHA are sensitive to oxidation during fish storage and processing, post-mortem degradation in the first hours following fish death is poorly documented. Here, we stored fish at two temperatures (2-4°C and 18-20°C) and monitored EPA+DHA content in dorsal fillet 6, 12 and 24 hours after fish death and after cooking (grill or steam). Storage duration was the only influencing factor, and EPA+DHA loss was faster at 18-20°C. Six hours after fish death, EPA+DHA content decreased by 1.3±1.3 mg.g-1 (9.6±9.5% of the initial content) but it was highly variable among individuals. Handling between fishing and storage should be as short and as cool as possible to preserve EPA+DHA and food safety. Regarding cooking, EPA+DHA and mono-unsaturated fatty acids increased in grilled fillets.

Highlights

► Storage duration had a higher impact than storage temperature on EPA+DHA content in mackerel dorsal fillet. ► EPA+DHA and mono-unsaturated fatty acids content increased in grilled fillets. ► EPA+DHA losses in mackerel dorsal fillet were highly variable among individuals.

Keywords : Oxidation, Fatty acids, Lipids, Small pelagic fish, Storage, Cooking method

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1. Introduction

Long-chain n-3 polyunsaturated fatty acids, and especially eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are essential lipids for several vital functions in human (Siriwardhana et al., 2012; Swanson et al., 2012). Due to insufficient *de novo* synthesis capacities, a daily dietary EPA+DHA intake of about 300 mg is recommended for healthy adults (FAO/WHO, 2010; Plourde & Cunnane, 2007). Several human populations however, face an EPA+DHA deficiency, caused by malnutrition or undernutrition (Stark et al., 2016). EPA and DHA human supplies are mainly ensured by the consumption of marine fish, which obtain these lipids through the food web, mainly from primary producers. Due to climate changes that may induce modifications in the physiology and community composition of primary producers (Galloway & Winder, 2015), the global EPA+DHA production is predicted to decrease (Hixson & Arts, 2016). Such decrease, combined with human population growth, might cause a shortage in EPA+DHA availability that may become a challenge for human food security in the next decades (Hicks et al., 2019). Preservation of EPA+DHA in food is thus of high importance.

Small and medium pelagic fish, also called blue-backed fish, play an important role in food security, as they are cheaper than large fishes and rich in nutrients, including EPA+DHA (Kawarazuka & Béné, 2011). Among them, the Atlantic mackerel *Scomber scombrus* is a fatty fish occurring in cold and temperate regions of the northern Atlantic Ocean, where it forms large schools near the surface to feed on zooplankton and small fishes. About one million tons of Atlantic mackerel are fished yearly, mainly by purse seine or pelagic trawl (FAO Fisheries & Aquaculture, 2020). Mackerels are generally frozen or chilled whole at collection, for further processing ashore (Sone et al., 2019). Storage method and duration, fish processing, and cooking procedures are critical steps for fish EPA+DHA preservation, because EPA and DHA are highly sensitive to oxidation (Gladyshev & Sushchik, 2019; Secci & Parisi, 2016). In the case of mackerel, whose lipid content can vary by a factor three among individuals, storage

temperature is among the main factor determining a successful storage (Romotowska et al., 2017). Studies have investigated lipid changes in long-term storage of mackerel for a large panel of storage temperatures and duration, from -27°C to +26°C and from a day to a year (Otero et al., 2019; Oucif et al., 2012; Romotowska et al., 2016, 2017; Standal et al., 2018). However, as for many other fish species, initial reference values were based on fish obtained from commercial fishing and the exact time of death was not well documented. Consequently, having information on the EPA+DHA changes in the first *post-mortem* hours could help detecting EPA+DHA losses during the first processing steps of fish (e.g., between fishing and the processing plants).

The objectives of this study were to quantify in Atlantic mackerel (i) EPA+DHA changes during the first *post-mortem* hours according to storage temperature; and (ii) the influence of the cooking method (grill and steam) on the EPA+DHA content. Fatty acids, including EPA+DHA, were quantified in the fillet of freshly euthanized mackerel, and at 6, 12 and 24 hours from death, on fish stored at two contrasted temperatures (2–4°C and 18–20°C).

2. Material & Method

2.1. Sample collection

Fifteen mackerels *Scomber scombrus* (25.2±3.3 cm in fork length) were collected by hand line in the Baie de Douarnenez, France on July 26th 2020. Seawater parameters were 16.2°C and 34.8 PSU, and air temperature was 18-20°C throughout the fishing operation. Fish were immediately euthanized by immersion in a 1 mL.L^{-1} eugenol bath (Fili@vet Reseau Cristal, France) for 5-10 min, then immediately labelled for later identification, and dissected on board. Sampling was restricted to the dorsal fillet from the right side of the fish (i.e., white muscle under the first dorsal fin).This area is homogenous in both lipid content and lipid oxidation in *S. scombrus* (Icekson et al., 1998), and in lipid and fatty acids contents in the related species

S. japonicus and *S. australasicus* (Bae et al., 2010). In a previous estimate conducted on three Atlantic mackerels kept frozen for 8 months at -15°C, the EPA+DHA contents varied from ca. 7 % among the sampled positions (results not shown). For each fish, about 0.5 g wet weight (ww) of dorsal white muscle was sampled without skin, flash frozen in liquid nitrogen, and stored in a dry shipper frequently refuelled with liquid nitrogen (T_0 sample). The total time between fish death and sample flash-freezing was less than 10 min. Fishes were then kept whole (neither eviscerated, nor filleted), and randomly stored either (i) at about 4°C in an insulated styrofoam box cooled with -20°C ice packs (n=8 fish), or (ii) at ambient temperature in a styrofoam box (n=7 fish). To consider the inter-individual variability, measurements were repeated over time on the same individuals. Six hours after the first tissue sampling, another 0.5 mg ww sample of dorsal muscle was taken on each fish next to the previous one (T_6) (Fig.1). Samples were flash frozen and stored in a dry shipper, and fishes were stored back in their respective box (i.e., ice packs or ambient). The same procedure was repeated 12 and 24 hours after the first tissue sampling (T_{12} and T_{24}, respectively) (Fig.1). Air temperature of the storage boxes (i.e., ice cold or ambient temperature) was recorded before each tissue sampling and ranked 2–4°C and 18–20°C throughout the storage test, respectively.

After the last sampling, dorsal fillet from the left side was filleted and cooked on ethanol-cleaned equipment, with neither oil nor condiment. Fillets with skin were either (i) grilled on a griddle at 80–90°C for five minutes, or (ii) steamed with 130 mL of tap water brought to a boil for 15 minutes (for each cooking method, half individuals were from each storage temperatures; Fig.1). European populations commonly use these two methods, steaming being particularly preferred for babies. After cooking, about 0.5 mg ww of fillet without skin was collected, flash frozen, and stored in a dry shipper. All the samples were then stored at -80°C for 40 days before subsequent analyses.

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99 2.2. Moisture analysis

100 Moisture was determined by gravimetry. Samples were weighed before and after a 65-hours
101 freeze-drying (Christ Alpha 1-2 LD plus lyophilizer). The mean analytical variability was 1%.
102 Immediately after freeze-drying, samples were homogenized with ball mill (Restch MM400)
103 and stored back at -80°C for four days before lipid extraction. Moisture was expressed in
104 percentage of wet weight.

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106 2.3. Fatty acid analysis

107 Lipids were extracted from ca. 60 mg of dry powder with 6 mL of solvent mixture
108 (CH_3Cl_3:MeOH, 2:1, v:v) directly added into glass vials (Sardenne et al., 2019). Extracts were
109 flushed with nitrogen gas, vortexed, sonicated for 15 min, and stored for 15 days at -20°C.
110 Tricosanoic acid (23:0) was added as internal standard to lipid extract. Lipids were then
111 transesterified with H_2SO_4 (3.8% in MeOH) at 100°C for 10 min. Fatty acid methyl esters
112 (FAME) were separated and quantified on a Varian CP8400 gas chromatograph (GC) equipped
113 in parallel with a Zebron ZB-WAX and a ZB-5HT column (both 30 m length, 0.25 mm internal
114 diameter, 0.25 µm film thickness; Phenomenex) and flame ionisation detectors at the
115 Lipidocean core facility, University of Brest, France. GC parameters were set as in Sardenne et
116 al. (2019). FAME were identified by comparing sample retention times to those of commercial
117 standard mixtures (Supelco 37-component FAME mix, BAME, and PUFA no. 1 and 3 mixes;
118 Sigma-Aldrich) using Galaxie 1.9.3.2 software (Varian). FAME content was converted into
119 fatty acids (FA) content based on 23:0 recovery. Total FA content was calculated as the sum of
120 41 identified FA (16 poly-unsaturated FA (PUFA), 15 mono-unsaturated FA (MUFA), and 10
121 saturated FA (SFA)). Data were expressed in $mg.g^{-1}$ dry weight to avoid confounding change
122 in lipid content with change in water content. The mean analytical variability was 4.2% for the
123 GC and 11.9% for the whole FA analysis.

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2.4. Data analysis

Contents in moisture, total FA, EPA+DHA, and FA families (SFA, MUFA, PUFA) were compared among storage temperatures, durations and their interaction using semi-parametric MANOVAs for repeated measures based on a central χ^2-distribution, which do not assume multivariate normality nor covariance homogeneity (RM function from the 'MANOVA.RM' package; Friedrich et al., 2018). Non-parametric paired Wilcoxon tests (V statistic) were used to refine differences between duration modalities. Unpaired Wilcoxon test (W statistic) were used to test for differences between storage temperatures at each duration, and between cooking modes. Spearman correlation tests (S statistic) were used to test for correlation between EPA+DHA losses at T_6, T_{12}, and T_{24}, and FA content at T_0, and linear regressions were used to test for fish length influence on FA contents. Data were analysed using R software 3.5.0 (R Development Core Team et al., 2018), and 'stats' and 'MANOVA.RM' packages.

3. Results

Initial EPA+DHA content was 16.2±4.0 mg.g^{-1} dw), representing about 30% and 80% of total FA and PUFA, respectively. The amount of FA varied as a function of fish length, with larger individuals having a higher content of total FA and EPA+DHA (i.e. from 12.2±0.2 to 17.6±0.6 mg.g^{-1} between 20 and 30 cm in fork length ; Fig 2). Biochemical contents of fish, including EPA+DHA (Table 1), changed with storage duration, but was not affected neither by storage temperature nor by the interaction between storage temperature and duration (Table 2). Changes in EPA+DHA content between storage conditions were highly variable among individuals (large SD; Table 1).

Regardless of the storage temperature, total FA content decreased between T_0 and T_{24}, from 60.9±21.8 to 51.0±24.1 mg.g^{-1} ($V=114$, $p<0.001$; Table 1, Fig. 3b). Variability in total FA

content (estimated through coefficients of variation) was lower at 2–4°C than at 18–20°C (T_6: 32.2% and 42.8%, T_{12}: 44.0% and 49.9%, and T_{24}: 40.1% and 58.0% at 2–4°C and 18–20°C, respectively). Regarding cooking, total FA content did not change from T_{24} in either grilled or steamed fillets (W=42, p=0.12; Fig. 3b).

EPA+DHA content decreased between T_0 and T_6 (V=110, p<0.01): paired differences indicated a loss of 1.3±1.3 mg.g^{-1} of the initial EPA+DHA content (0.9±1.3 and 1.9±1.1 mg.g^{-1} at 2–4°C and 18–20°C, respectively), equating to a loss of 9.6±9.5% (5.5±8.1% and 14.2±9.3% , respectively). EPA+DHA content also decreased between T_6 and T_{12} (V=103, p<0.05), but not after (V=61, p=0.98; Fig. 3c). No correlations between total FA content at T_0 and EPA+DHA losses at T_6, T_{12}, and T_{24} were detected (S=814, p=0.10; S=450, p=0.48; S=438, p=0.43, respectively). Regarding cooking, grilled fillet gained 3.4±3.5 mg.g^{-1} and steamed fillets lost 0.2±3.4 mg.g^{-1} of EPA+DHA from T_{24} (W=46, p<0.05; Table 1). As for EPA+DHA, PUFA, MUFA and SFA contents decreased between T_0 and T_6 (all p<0.01; Fig. 3d to f), but only PUFA continued to decrease between T_6 and T_{12} (V=98, p<0.05). Cooking methods affected both EPA+DHA and MUFA contents, which were already lowered by storage (W=46, p<0.05 and W=47, p<0.05): EPA+DHA and MUFA gained 3.4±3.5 and 5.7±5.4 mg.g^{-1} in grilled fillet and lost 0.2±3.3 and 2.3±6.3 mg.g^{-1} in steamed fillet, respectively (Fig. 3c and e).

4. Discussion

EPA and DHA are sensitive to oxidation, but their loss during fish processing in the first *post-mortem* hours of the Atlantic mackerel is not well documented. Here, we find that (i) storage duration was the only factor explaining FA losses in the first hours after fish death, and (ii) grilled fillet had higher EPA+DHA content than steamed fillet. However, changes in EPA+DHA content were highly variable among individuals, probably in relation to other sources of variability (e.g. sampling position, analytical variability).

High inter-individual variability was observed both in FA contents and in FA losses. Fish length explained most of the variability in the initial FA content, and storage duration was the only significant factor explaining FA losses, despite faster FA losses at the high storage temperature (18–20°C). Other sources of variability in FA losses might be the heterogeneity in FA compositions among tissue sampling positions, despite the fact that all positions were close to each other, i.e., dorsal white muscle under the first dorsal spine. While the natural variability of EPA+DHA content in the dorsal fillet of frozen Atlantic mackerel was relatively low (ca. 7%), it remains to be assessed for fresh individuals. Regarding changes due to storage duration, FA contents decreased only six hours after fish death with: 9.6±9.5% of the initial EPA+DHA content been lost. Again, the large inter-individual differences observed can be explained by the sources of variability previously mentioned (i.e., sampling position and analytical variability). However, only PUFA continued to decrease 12 hours after death. PUFA are generally considered as the FA the most sensitive to degradation, especially to oxygen contact that causes peroxidation (Couturier et al., 2020). While lipid hydrolysis tends to increase with fish fat content (Aubourg et al., 2005; Rudy et al., 2016), this relationship has not been reported for mackerel, even over several storage months (Aubourg et al., 2005). Similarly, we did not observe any relationship between the initial fish FA content and EPA+DHA losses in the first *post-mortem* hours of mackerel. These results highlight the importance of fast handling, and to a lesser extent of cool handling, even before the *rigor mortis* that usually occurs at about 18-21 hours *post-mortem* in mackerel (Anders et al., 2020). Although temperature did not play a crucial role for lipid oxidation at the time scale of this study (24 hours), it is relevant for other degradation mechanisms such as hydrolysis or histamine production (Couturier et al., 2020; Zou & Hou, 2017). Avoiding filleting, favoring fast frozen of fish, as well as the maintenance of low temperature throughout long-term storage could save highly valuable FA in the Atlantic mackerel (Aubourg et al., 2005; Romotowska et al., 2017), caveat that can probably be

generalized to other fatty and small pelagic fishes (e.g., Rudy et al., 2016). This procedure is also relevant for scientific samplings of fish obtained from professional fisheries or from remote areas, which could rapidly loose FA before analysis, including EPA+DHA, due to a lack of proper storage.

Regarding cooking methods, grilled fish had higher FA contents, especially EPA+DHA and MUFA, than steamed fish, as well as than T_0 fish (all in dry weight). A higher FA content measured after grilling might be related (i) to changes in the fillet texture that might improve FA extraction from tissue, as high heating is commonly used by the industry for fish oil extraction (Adeoti & Hawboldt, 2014); or (ii) to FA exchanges with the subcutaneous fat beneath the skin (fillets were cooked with the skin), favoured by the increasing temperature. Studies have shown no exchanges of FA between fillet and skin of the Atlantic mackerel during iced and frozen storage (Xing et al., 1993), but extensive exchanges can occur during frying (Sebedio et al., 1993). In addition, the skin of small pelagic fish is relatively rich in EPA+DHA: about 19%, 26%, 25% and 13% of total FA for the Atlantic mackerel, *Rastrelliger kanagurta*, *Sardinella maderensis* and *Sardinella aurita*, respectively (Njinkoué et al., 2002; Sahena et al., 2010; Zuta et al., 2003). This suggests that the skin should be kept to cook Atlantic mackerel fillets. Extended tests including other cooking methods should however be conducted to determine the best cooking method to preserved the valuable EPA+DHA from the Atlantic mackerel. Indeed, the summer Atlantic mackerel is an excellent food source of EPA+DHA: with about 4 to 5 $mg.g^{-1}$ in wet weight, a daily consumption of 75 g (raw or steamed) or 60 g (grilled) of dorsal fillet would be enough to cover the EPA+DHA daily requirements in adults.

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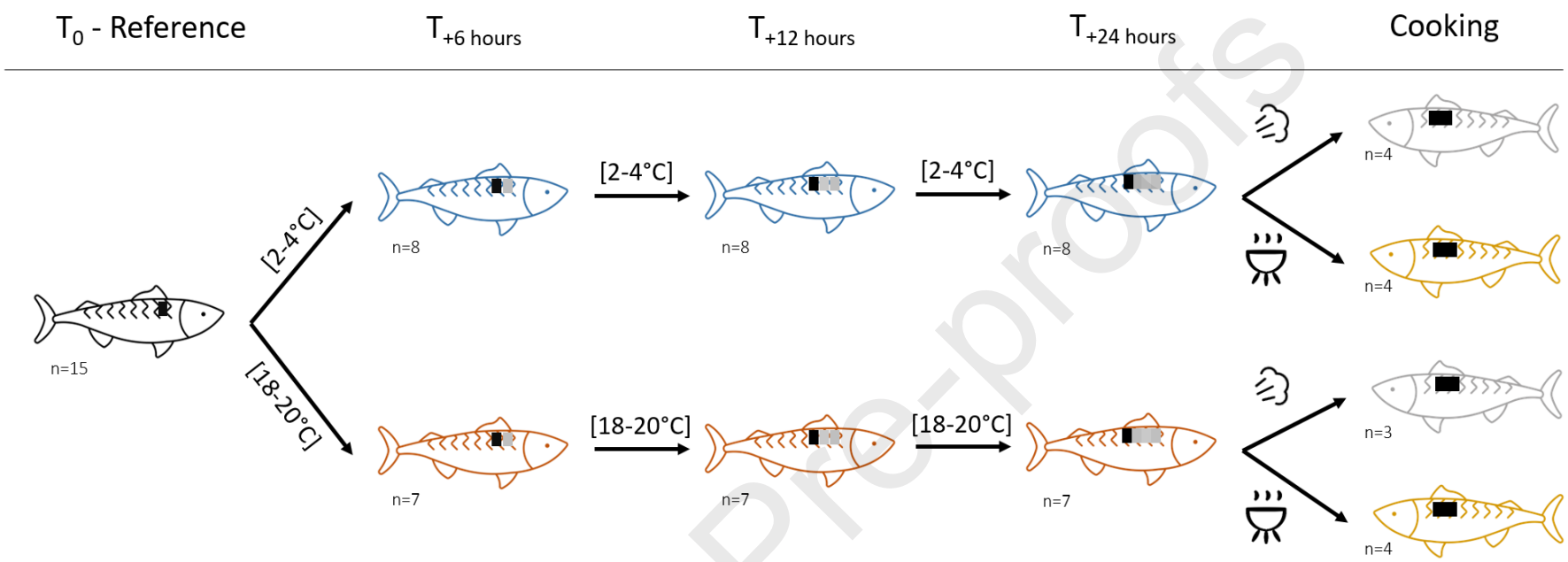

Fig. 1 | Outline of the sampling design testing for the influence of storage temperature (2-4°C and 18-20°C), storage duration (from T_6 to T_{24} hours), and cooking method (grill and steam) on the fatty acid content of the Atlantic mackerel *Scomber scombrus*. Dark squares indicate the muscle sampling position at each step, and the grey ones the previously sampled positions. For cooking, we sampled the left side of the fish.

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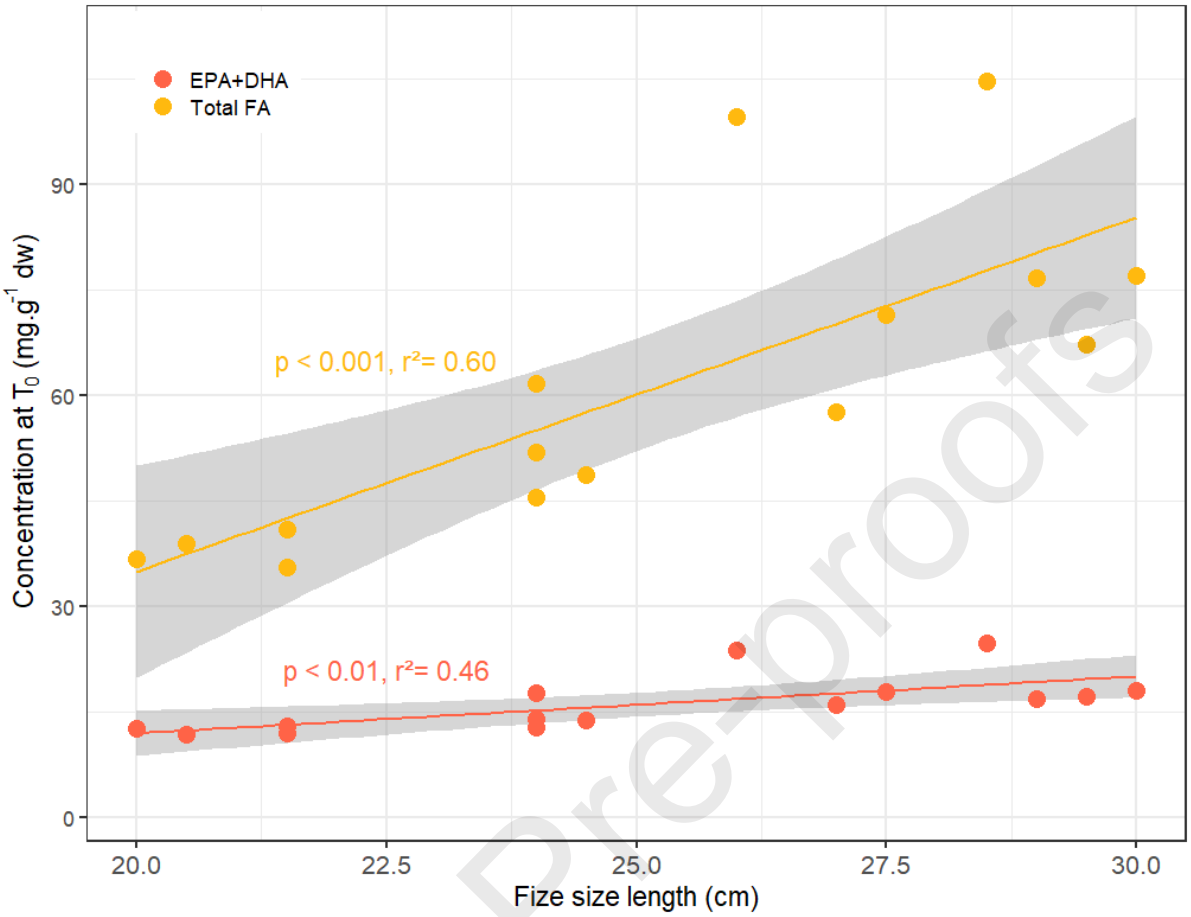

Fig. 2 | Total fatty acids (FA) and EPA+DHA contents (in $mg.g^{-1}$ of dry weight) in the dorsal fillet of Atlantic mackerel *Scomber scombrus* as a function of fish fork length. Fish (n=15) were collected from the Baie de Douarnenez, France, in July 2020. Grey areas are standard errors for the linear regressions.

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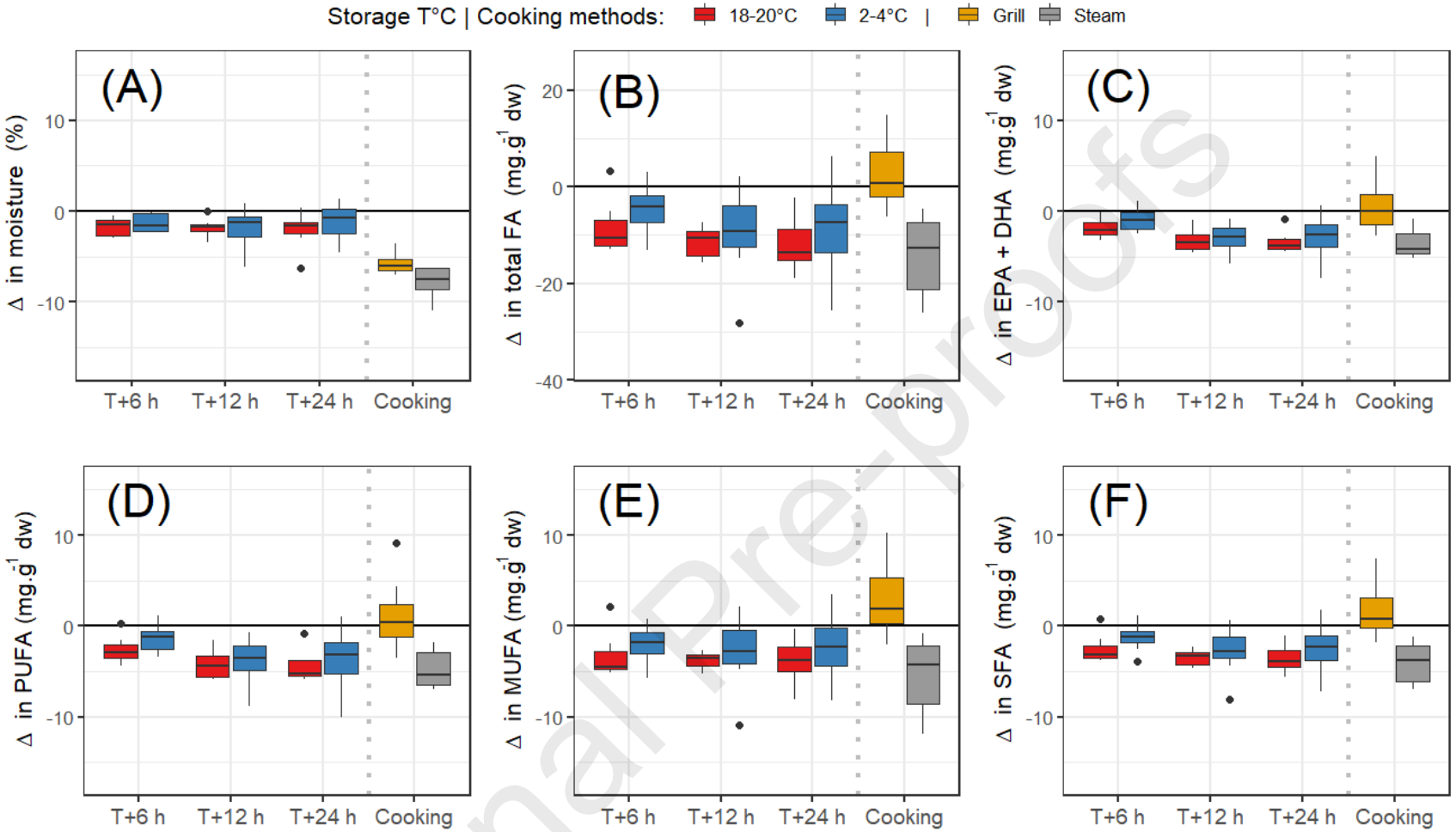

Fig. 3 | Boxplots of differences from initial values (T_0 = at fish death) for dorsal fillet contents in (A) moisture , (B) total fatty acids (FA), (C) EPA+DHA, (D) Poly-unsaturated FA (PUFA), (E) Mono-unsaturated FA (MUFA), and (F) Saturated FA (SFA) during the first post-mortem hours (T_6 to T_{24}) and cooking of Atlantic mackerel, according to storage temperature and cooking methods (display by colour). In each boxplot, the thick black bar represents the median value, the box contains 50% of the data, and dots are outliers.

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Table 1 | Mean ± standard deviation for moisture in % per wet weight, total fatty acids (FA), EPA+DHA, poly-unsaturated FA (PUFA), mono-unsaturated FA (MUFA), and saturated FA (SFA) contents in $mg.g^{-1}$ dry weight (dw), from the dorsal fillet of Atlantic mackerel after three storage durations (T_6, T_{12}, and T_{24} hours) at two storage temperatures (2-4°C and 18-20°C), and after two cooking methods (grill and steam). N is the number of individuals.

	Reference	Storage test						Cooking method	
	T_0	T_6		T_{12}		T_{24}			
		[2-4°C]	[18-20°C]	[2-4°C]	[18-20°C]	[2-4°C]	[18-20°C]	Grill	Steam
N	15	8	7	8	7	8	7	8	7
Moisture (%)	75.4 ± 3.0	73.5 ± 2.9	74.3 ± 3.4	72.9 ± 4.3	74.3 ± 3.3	73.7 ± 3.2	74.0 ± 4.7	69.2 ± 2.7	68.3 ± 3.1
Total FA ($mg.g^{-1}$ dw)	60.9 ± 21.8	60.6 ± 19.5	48.0 ± 20.5	55.6 ± 24.4	44.8 ± 22.3	56.7 ± 22.7	44.5 ± 25.8	66.5 ± 21.0	46.9 ± 19.7
EPA+DHA ($mg.g^{-1}$ dw)	16.2 ± 4.0	15.9 ± 3.6	13.6 ± 3.8	13.8 ± 4.3	12.3 ± 4.4	14.0 ± 4.0	12.1 ± 5.2	16.5 ± 4.2	12.9 ± 4.1
PUFA ($mg.g^{-1}$ dw)	20.6 ± 5.6	20.3 ± 5.3	17.0 ± 5.2	17.7 ± 6.1	15.4 ± 5.9	17.9 ± 5.5	15.3 ± 7.0	21.6 ± 6.0	16.3 ± 5.6
MUFA ($mg.g^{-1}$ dw)	17.9 ± 10.1	18.0 ± 9.3	12.4 ± 9.3	17.1 ± 11.6	11.8 ± 9.9	17.6 ± 11.0	11.8 ± 11.2	20.8 ± 9.2	12.3 ± 9.2
SFA ($mg.g^{-1}$ dw)	16.2 ± 6.3	16.0 ± 5.5	12.3 ± 6.0	14.6 ± 7.0	11.4 ± 6.5	14.9 ± 6.4	11.3 ± 7.5	17.8 ± 6.0	12.1 ± 5.5

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Table 2 | Summary of results obtained from repeated MANOVA to test the influence of storage temperature, duration and their interaction on biochemical contents of Atlantic mackerel dorsal fillet (moisture, total fatty acids (FA), EPA+DHA, poly-unsaturated FA (PUFA), mono-unsaturated FA (MUFA), and saturated FA (SFA)).

	χ^2	df	p value
Moisture			
Storage temperature	0.3	1	0.580
Storage duration	35.5	3	<0.001
Storage temperature * duration	2.4	3	0.530
Total FA			
Storage temperature	0.9	1	0.332
Storage duration	68.6	3	<0.001
Storage temperature * duration	2.9	3	0.400
EPA+DHA			
Storage temperature	0.7	1	0.409
Storage duration	127.4	3	<0.001
Storage temperature * duration	3.6	3	0.305
PUFA			
Storage temperature	0.8	1	0.384
Storage duration	101.0	3	<0.001
Storage temperature * duration	3.3	3	0.342
MUFA			
Storage temperature	1.0	1	0.318
Storage duration	44.8	3	<0.001
Storage temperature * duration	2.2	3	0.533
SFA			
Storage temperature	1.0	1	0.328
Storage duration	73.1	3	<0.001
Storage temperature * duration	4.0	3	0.266

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Author credits

Fany Sardenne: conceptualization, methodology, software, investigation, writing – original draft, review & editing. **Eleonora Puccinelli**: investigation, writing – review & editing. **Marie Vagner**: writing – review & editing, funding acquisition. **Laure Pecquerie**: writing – review

& editing, funding acquisition. **Fabienne Le Grand**: validation, resources. **Antoine Bideau**: methodology. **Philippe Soudant**: validation, writing – review & editing, funding acquisition.

Declaration of 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Storage duration had a higher impact than storage temperature on EPA+DHA content in mackerel dorsal fillet
- EPA+DHA and mono-unsaturated fatty acids content increased in grilled fillets
- EPA+DHA losses in mackerel dorsal fillet were highly variable among individuals

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