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Differences in nutrient and undesirable substance concentrations in *Maurolicus muelleri* across the Bay of Biscay, Norwegian fjords, and the North Sea

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Introduction: We are having pressing issues of global food insecurity and malnutrition. Mesopelagic communities in the North Atlantic have been estimated to have high biomasses of organisms. Some of these low-trophic organisms are known to be nutrient-dense and may thus contribute to food security and nutrition. Here, we aim to understand the variation in nutrient and undesirable substance concentrations in a common mesopelagic species, *Maurolicus muelleri* in the North Atlantic Ocean.

Methods: We sampled the *M. muelleri* from the Bay of Biscay (BB), Norwegian fjords (NF), and the North Sea (NS). The concentrations of micro- and macronutrients, undesirable metals, and persistent organic pollutants (POPs) were measured in composite whole fish samples.

Results: We found no difference across the sampling areas in the selected micronutrients except that the NF and NS samples had higher vitamin A1 concentrations than the BB samples. The NF samples had higher concentrations of fat, fatty acids, and POPs but lower concentrations of cadmium than the BB and NS samples; the differences in fat and fatty acids were only marginal in the NF-BB pair. The BB samples had lower arsenic concentrations than the NS samples, and lower concentrations of erucic acid and mercury than the NF and NS samples. Comparing the measured values against existing EU regulation values for nutrients and undesirable substances for human consumption, we found that the samples from NS and BB may cause food safety concerns due to their high cadmium concentrations, while the *M. muelleri* from all the sampling areas are qualified as good sources of iron, selenium, vitamin A1, and ω -3 fatty acids.

Discussion: This study confirms that *M. muelleri* from the North Atlantic Ocean may play an important role in food security and nutrition. However, potential variations in nutrient and undesirable substance concentrations related to

seasonality, fish body size, and maturity level shall be taken into consideration prior to exploiting such a marine resource. Further understanding of trophic ecology, life cycles, and productivity of *M. muelleri* is essential to investigate the drivers behind the observed variation in nutrient and undesirable substance concentrations.

KEYWORDS

mesopelagic, food security, food safety and quality, nutrient, contaminant, marine resource

Introduction

Global food security remains under challenge to date (FAO, 2022; Abay et al., 2023; Alabi and Ngwenyama, 2023), and malnutrition is one of the most pressing issues (FAO, 2022; Stevens et al., 2022), particularly in sub-Saharan Africa, southern Asia and the Caribbean (Zurayk, 2020; FAO, 2022). Approximately one in two children under five years of age suffer from deficiencies in vitamins and other essential nutrients globally (UNICEF, 2019). Deficiencies in certain essential nutrients may cause certain diseases. Iron deficiency-induced anaemia (Camaschella, 2015; Stevens et al., 2022), iodine deficiency disorders (Pearce and Zimmermann, 2023), and vitamin A deficiency-induced eye diseases (Rice et al., 2004; Zhao et al., 2022) are still prevalent globally or regionally.

Aquatic foods are recognised as one of the key solutions to combat global hunger and malnutrition (Smith et al., 2010; Tacon and Metian, 2013; Hicks et al., 2019; Costello et al., 2020; Golden et al., 2021; Cai and Leung, 2022). However, current aquatic food systems are challenged by multiple global and regional threats, including policies (e.g. trade policies, Chan et al., 2019), trade (Smith et al., 2010), climate change (Tanentzap et al., 2020; Tigchelaar et al., 2021), pollution (Hallgren et al., 2014), overfishing (Jackson et al., 2001; Scheffer et al., 2005) and post-harvest loss (Ahmed, 2008; Aulakh et al., 2013). Generating knowledge of aquatic foods may help address some of these challenges. Nevertheless, new marine resources of high quality and quantity can contribute to combating global hunger and malnutrition.

Mesopelagic communities are one of the least studied marine systems compared to others with commercial interests. However, it is suggested that they may play an important role in food security and nutrition (FSN) (Standal and Grimaldo, 2020; Kourantidou and Jin, 2022; Fjeld et al., 2023). Mesopelagic fishes are often low in trophic level and diverse (Eduardo et al., 2022) and are estimated to contain more than ten billion tons of biomass globally (Irigoién et al., 2014; Standal and Grimaldo, 2021). In addition, some mesopelagic species have been found to be dense in essential nutrients such as iodine, selenium, vitamin A₁ and ω-3 fatty acids (e.g. Alvheim et al., 2020; Grimaldo et al., 2020; Voronin et al., 2023). Aquatic foods are a better source of a range of micronutrients than other common animal-based foods, and an increase in the intake of aquatic foods has been shown to have a major impact on

public health (Golden et al., 2021). This evidently points to the potential of utilising mesopelagic species as feed and for human consumption (Fjeld et al., 2023). However, nutrient concentrations in wild-caught seafood often vary greatly geographically (Hicks et al., 2019), and relevant data for mesopelagic species are lacking. This may have implications for potential utilisation of mesopelagic species.

Undesirable substances in aquatic food are yet a concern (e.g. García et al., 2000; Bank et al., 2020) because they can cause human health issues (Qin et al., 2010; WHO, 2010; Ruzzin, 2012; Shi et al., 2019). These undesirable substances include microplastic (Lusher et al., 2016; Barboza et al., 2018), metals and metalloids (e.g. mercury, arsenic, and lead), and persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), polybrominated flame-retardants (PBDEs), dioxins and furans (Corsolini et al., 2005; Panseri et al., 2019; Wiech et al., 2020). Some mesopelagic species have been shown to have relatively low undesirable substance concentrations (Wiech et al., 2020; Berntssen et al., 2021). Yet, potential differences in undesirable substance concentrations have been observed among some mesopelagic species (e.g. Wiech et al., 2020). This may intrinsically result from the differences in the basal undesirable substance concentrations (Islam and Tanaka, 2004; Beiras, 2018). Thus, further investigation to infer the risks and benefits of exploiting mesopelagic species for FSN is required.

The North Atlantic Ocean provides valuable fishing grounds for many countries to nourish regional and global populations (Pauly and Maclean, 2003). While most of the local fisheries focus on the traditional and/or commercial species (Rose, 2007), mesopelagic species remain as an opportunity to increase the productivity of aquatic food (Grimaldo et al., 2020). *Maurolicus muelleri* is one of the most abundant mesopelagic species in the North Atlantic Ocean (Salvanes and Stockley, 1996; Salvanes and Kristoffersen, 2001; Rees et al., 2020). Its biomass estimation in Southern Norway and West of the British Isles was conducted in 1971–1976 with both echosounders and trawls, which resulted in a stock size of between 20,000 and 1,600,000 tons (Gjøsæter, 1986). Recently, several trial fisheries also suggested the high biomass of *M. muelleri* in Iceland (46,000 and 18,000 tons in 2009 and 2010, respectively; Standal and Grimaldo, 2021), the North Sea (1,500 tons of mostly *M. muelleri*; Bjordal and Thorvaldsen, 2020), and the Bay of Biscay (70,000 to 160,000 tons in 2014–2017; Sobradillo et al., 2019). To assess the potential of fisheries of *M. muelleri* to

contribute to FSN, it is essential to understand their quality (nutrients against undesirable substances) and how this may vary within the North Atlantic Ocean.

In this study, we aimed to investigate the concentrations of nutrients and undesirable substances in *M. muelleri* from different sampling areas in the North Atlantic Ocean to examine potential geographic variations. Specifically, we analysed the concentrations of key nutrients and undesirable substances in the *M. muelleri* from three sampling areas (Bay of Biscay, Norwegian fjords, and North Sea); compared these concentrations with EU regulation values for nutrients and undesirable substances in food; and detected any differences in these values across the sampling areas.

Materials and methods

Sample collection

The Institute of Marine Research Norway (IMR) conducted mesopelagic trawls in Norwegian fjords (NF, Figure 1; including Bjørnafjorden, Boknafjorden, and Osterfjorden) during December 2018 (data published in Alvheim et al., 2020, and Wiech et al., 2020), March 2020 and May 2020, and in the North Sea (NS, Figure 1) during March 2020. Two pelagic otter trawls with equal-sized mesh, one 35 m² and one 350 m² openings were deployed (Table 1). AZTI conducted mesopelagic trawls in the Bay of Biscay (BB, Figure 1) during September 2019 and September 2020 using a Gloria HOD 352 pelagic trawl (Table 1). After landing, *M. muelleri* individuals were identified, and a subsample of the catch (approximately 100 individuals in BB and at least 50 in NF and NS) was used to estimate the size distribution of the catch by measuring the standard length. All samples were then frozen on board at -18 °C and kept in the dark to avoid potential degradation of light-sensitive compounds (e.g. vitamins).

Substance analyses

From each trawl, we made one or two composite sample(s) by selecting and homogenising at least 25 whole *M. muelleri* individuals. We analysed important substances (including both nutrients and undesirable substances) based on existing relevant studies (i.e. Alvheim et al., 2020; Wiech et al., 2020) and EU regulations on nutrients and undesirable substances (for all analysed substances, see Appendix 1) with analytical methods (Table 2) which are accredited according to NS-EN ISO/IEC 17025 (2017) or are holding the status of National Reference Laboratory (NRL).

Data analysis

We first screened the data. For nutrients with less than 25% of measured values below the limit of quantification (hereafter <LOQ) for all the sampling areas, we chose the upper bound (UB) values for the data analysis, i.e. the LOQ value. Otherwise, we excluded the substance with more than 25% of values <LOQ for at least one

sampling area (e.g. vitamin A₂ and vitamin D₃). For all undesirable substances with measured values <LOQ, we used the UB values. The data collected in NF were from three different months (Table 1), they were pooled together due to no statistically significant difference found across the sampling months except for arsenic (Appendix 2).

To decide whether the measured nutrient concentrations could be defined as a source of a given nutrient, we compared them with existing EU regulation values (i.e. thresholds). For minerals and vitamins, we adopted EU Regulation 1169/2011 (<https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:en:PDF>). The threshold for a significant source of a given micronutrient refers to whether the product contains 15% or more of the nutrient reference values (NRVs) per 100 g. Here, we calculated these thresholds to concentrations by $15\% \times \text{NRV} \div 100\text{g}$. For macronutrients, which only included fatty acids (FAs) in this study, we compared the total ω -3 FAs (expressed as the sum of eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) with the EU Nutrition Claim in EU Regulation 1047/2012 (https://food.ec.europa.eu/safety/labelling-and-nutrition/nutrition-and-health-claims/nutrition-claims_en), which included a threshold for a source of ω -3 FAs, and a threshold that the food item contains high ω -3 FAs.

To decide whether the measured concentration of an undesirable substance was low/high risk to human consumers for a given undesirable substance, we compared them with the maximal levels (MLs) for food established in EU Regulation 1881/2006 (<https://faolex.fao.org/docs/pdf/eur68134.pdf>).

To categorise the measured substance values from each sampling area, we compared the 25th and 75th quantiles with the thresholds and interpreted the categorisation accordingly (Table 3). Because the sample size of substance values for each sampling area was relatively low, we did not assume a normal distribution of the substance values. Thus, to detect differences across sampling areas for each substance, we compared the substance values using the non-parametric Wilcoxon test in Rstudio 1.4.1106 (Team, 2009). The difference between a pair of sampling areas was considered significant if *p*-value was below 0.05; and such a difference was considered marginal if *p*-value was not below 0.05 and the median value of one sampling area was outside of the 25th or 75th quantile of another sampling area (i.e. the box in the boxplot, Figure 2).

Results

We conducted four mesopelagic trawls in the Bay of Biscay (BB), nine in Norwegian fjords (NF), and eight in the North Sea (NS) (Table 1). In total, we collected four composite samples from BB, and ten from NF and NS ($n_{\text{Bay of Biscay}} = 4$, $n_{\text{Norwegian fjord}} = 10$, and $n_{\text{North Sea}} = 10$). The *M. muelleri* collected in BB had standard lengths (SLs) ranging from 25.19 ± 4.87 to 32.37 ± 3.56 mm; those collected in NF had SLs ranging from 23.57 ± 2.63 to 51.17 ± 11.47 mm; and those collected in NS had SLs ranging from 28.40 ± 7.90 to 50.60 ± 7.30 mm.

In total, we analysed three gross proximate, ten essential elements, three vitamins, 37 individual FAs, six hazardous metals

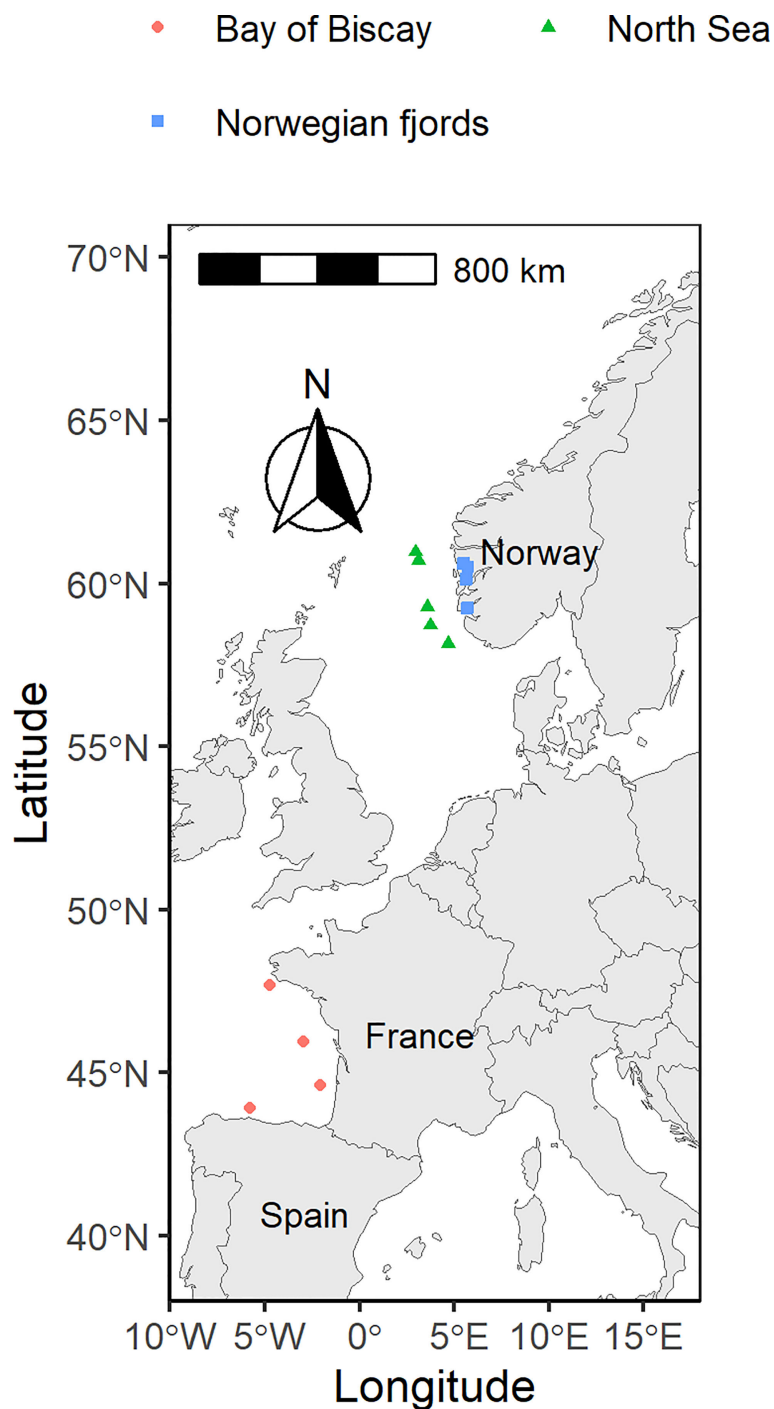


FIGURE 1

Map of sampling sites for each sampling area in the Bay of Biscay, Norwegian fjords, and the North Sea. Each dot represents the centre of the trawl transect.

and metalloids, 43 POPs, and the sum of all fatty alcohols (Appendix 1). We selected 22 substances while excluding two important nutrients, vitamin A₂ and vitamin D₃, from the analyses due to high percentages of values < LOQ (Table 4, Figure 2, Appendix 1).

In terms of gross proximate, the *M. muelleri* from NF had a dry matter content ($31.89 \pm 1.90\%$) statistically significantly higher than

that from NS ($24.36 \pm 1.10\%$); and that from NS was similar to that from BB ($25.78 \pm 1.92\%$; Table 4, Figure 2). Similar trends were found in fat content (NF, NS, and BB: $16.16 \pm 2.03\%$, $6.48 \pm 1.34\%$, and $6.40 \pm 1.82\%$, respectively; Table 4, Figure 2), while completely opposite trends were found in protein concentration ($10.83 \pm 1.39\%$, $16.32 \pm 0.62\%$, and $16.50 \pm 0.59\%$, respectively; Table 4, Figure 2).

TABLE 1 Mesopelagic sampling information of materials collected in this study, including sampling area, sampling period (month & year), the number of trawls for each period (n), net description, the depth, speed and duration of trawls.

Sampling area	Month & Year (n)	Net description	Depth (m) (mean ± SD)	Speed (knot)	Duration (minute)
Bay of Biscay	Sep. 2019 (2)	Gloria HOD 352 pelagic trawl with 15-m of vertical opening with a 10-mm mesh size (bar length) at the codend	145 ± 48	~3.8	~47
	Sep. 2020 (2)		121 ± 100	~3.6	~42
Norwegian fjords	Dec. 2018 (4)	Two pelagic otter trawls: a 35-m ² (3x3 mm ² mesh) and a 350-m ² aperture (7x7 mm ² mesh) nets	Depends on the occurrence of fish	~2-3	~30-180
	Mar. 2020 (2)				
	May 2020 (3)				
North Sea	Mar. 2020 (8)				

Micronutrients

There were statistically significant differences in the vitamin A₁ concentrations. The NF and NS samples had higher vitamin A₁ concentrations (1082.86 ± 133.84, and 900.00 ± 167.33 µg/100g [NF, and NS, respectively]; Table 4, Figure 2) than the BB samples (132.50 ± 37.50 µg/100g; Table 4, Figure 2), while no difference was observed in the other micronutrients across the sample areas (Table 4, Figure 2). Compared with relevant EU regulation values, the *M. muelleri* from all the sampling areas were shown to be significant sources of selenium/Se (51.00 ± 7.01, 50.10 ± 2.90, 62.10 ± 3.48, and 8.25 µg/100g [BB, NF, NS, and the regulation value, respectively]) and vitamin A₁ (132.50 ± 37.50, 1082.86 ± 133.84, 900.00 ± 167.33, and 120 µg/100g [BB, NF, NS, and the regulation value, respectively]); and the *M. muelleri* from BB and NS was a significant source of iron/Fe (2.13 ± 0.57, 3.84 ± 1.16, and 2.1 mg/100g [BB, NS, and the regulation value, respectively]; Table 4, Figure 2).

Macronutrients

The *M. muelleri* from NF had higher FA concentrations (e.g. EPA +DHA = 15.98 ± 2.02 mg/g; Table 4, Figure 2) than those from NS (statistically significant; e.g. EPA+DHA = 6.57 ± 0.61 mg/g) and BB (marginal; e.g. EPA+DHA = 10.17 ± 2.20 mg/g). The *M. muelleri* from all the sampling areas had EPA+DHA concentrations surpassing the relevant EU regulation value for 'source of ω-3 FAs' (0.8 mg/g; Table 4, Figure 2).

Undesirable substances

In wet weight, the *M. muelleri* from NF had statistically significantly higher concentrations of all selected POPs (e.g. PCDD/F = 0.93 ± 0.13 WHO-TEQ pg/g; Table 4, Figure 2) but lower concentrations of cadmium/Cd (0.03 ± 0.00 mg/kg) than those from BB (e.g. PCDD/F = 0.13 ± 0.02 WHO-TEQ pg/g, and Cd = 0.08 ± 0.01

TABLE 2 List of substance groups and analytical methods.

Substance	Analytical method	References to analytical method	Outcome expression
Trace metals and alkaline metals	Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)	Julshamn et al. (2007); Alvheim et al. (2020); Reksten et al. (2020); Wiech et al. (2020).	Concentration (mg/kg)
Vitamin A & D	High-performance liquid chromatography (HPLC)	CEN (1999); de Normalisation (2009); Alvheim et al. (2020); Reksten et al. (2020).	Concentration (mg/kg)
Dry matter		Alvheim et al. (2020).	Concentration (%)
Protein content (crude protein)		Biancarosa et al. (2017); Reksten et al. (2020).	Concentration (%) of protein was derived from the measured nitrogen content by multiplying it with the N-to-protein conversion factor 6.25 (Kjeldahl, 1883).
Fat content (crude fat)		Reksten et al. (2020).	Concentration (%)
Fatty acids	FS-direct 465, and FS SAMM 041	Meier et al. (2006); Alvheim et al. (2020).	Concentration (mg/g)
Dioxins, Furans, Polychlorinated Biphenyls, and Polybrominated Flame-Retardants	High-resolution gas chromatography/mass spectrometry (HRGC/MS)	Berntssen et al. (2021).	Dioxins and dl-PCBs: WHO-TEQ/g (using WHO-TEF 2005). The measured values were also corrected by fat content using: $POP_{corrected} = \frac{POP_{measured}}{Fat\ content}$

TABLE 3 Categorisation based on substance values against established relevant EU regulation values (i.e. threshold).

Measured values against thresholds	Categorisation	
	Nutrients	Undesirable substances
25 th quantile below the threshold	Not a sufficient source of the nutrient	Low risk
The threshold between 25 th and 75 th quantile	A significant source of a given micronutrient/a source of ω -3 fatty acids/contains high ω -3 fatty acids	Concerning level of risk
75 th quantile above the threshold		High risk

Note that the wording of categorisation for nutrients differs among nutrients.

mg/kg) and NS (e.g. PCDD/F = 0.46 ± 0.08 WHO-TEQ pg/g, and Cd = 0.08 ± 0.01 mg/kg). The samples from NS had higher concentrations of PCDD/F, dioxin+dl-PCB (0.70 ± 0.13 WHO-TEQ pg/g; Table 4, Figure 2), PBDE₇ (0.25 ± 0.04 ng/g), and arsenic/As (4.09 ± 0.19 mg/kg) than those from BB (dioxin+dl-PCB = 0.29 ± 0.06 WHO-TEQ pg/g, PBDE₇ = 0.07 ± 0.02 ng/g, As = 2.25 ± 0.35 mg/kg). The *M. muelleri* from NF and NS had higher mercury/Hg (0.03 ± 0.00 mg/kg, and 0.02 ± 0.00 , respectively; Table 4, Figure 2) and erucic acid concentrations (0.81 ± 0.05 mg/g, and 0.84 ± 0.03 mg/g, respectively) than those from BB (Hg = 0.01 ± 0.00 mg/kg, erucic acid = 0.08 ± 0.03 mg/g). After fat correction, the PCDD/F, dioxin+dl-PCB, and PBDE₇ levels were statistically significantly higher in the samples from NF (e.g. \blacktriangleright PCDD/F = 0.06 ± 0.01 WHO-TEQ pg/g; Table 4, Figure 2) and NS (e.g. \blacktriangleright PCDD/F = 0.07 ± 0.01 WHO-TEQ pg/g) than those from BB (e.g. \blacktriangleright PCDD/F = 0.02 ± 0.00 WHO-TEQ pg/g), while no difference was detected in PCB₆ (1.01 ± 0.38 , 0.36 ± 0.02 , and 0.38 ± 0.08 ng/g [NF, NS, and BB, respectively]) and PCB₇ levels (1.16 ± 0.44 , 0.41 ± 0.02 , and 0.42 ± 0.08 ng/g) across the sampling areas (Table 4, Figure 2).

Considering the established maximum levels (MLs), the *M. muelleri* from all the sampling areas had PCDD/F, dioxin+dl-PCB (0.29 ± 0.06 , 1.73 ± 0.23 , and 0.70 ± 0.13 WHO-TEQ pg/g [BB, NF, and NS, respectively]; Table 4, Figure 2), Hg, and lead/Pb concentrations (0.02 ± 0.00 , 0.05 ± 0.03 , and 0.03 ± 0.01 mg/kg) below the MLs for human consumption (Table 4, Figure 2). However, the Cd concentrations measured in the BB and NS samples exceeded the ML for human consumption (0.05 mg/kg; Table 4, Figure 2), assuming that the *M. muelleri* will be consumed whole due to its small size as a convention similar to other small fishes (Roos et al., 2007; Longley et al., 2014; Kolding et al., 2019).

Discussion

Our study confirmed the potential roles of *Maurolicus muelleri* from the North Atlantic Ocean for food security and nutrition by proving a novel nutrient-dense and low-risk aquatic food. However, there were statistically significant differences in the concentrations of several nutrients and undesirable substances across the sampling areas, which may influence the utilisation of this new marine resource.

Roles in nutrition provision

The *M. muelleri* from all the sampling areas in the North Atlantic Ocean were found to have several benefits. According to relevant EU regulations, they were qualified as significant sources of Se and vitamin A₁ and contained high ω -3 FAs; and those from BB and NS were qualified as a significant source of Fe.

Compared with other mesopelagic species in BB (Chouvelon et al., 2022) and assuming the dry matter content of *M. muelleri* is 25% (units are in dm, otherwise in ww), the *M. muelleri* from BB were rather low in the concentrations of Fe (Fe_{BB} = 85.20, Fe_{Others} = 47.9–333.3 mg/kg dm), Se (Se_{BB} = 2.04, Se_{Others} = 1.68–2.91 mg/kg dm), and Zn (Zn_{BB} = 51.20, Zn_{Others} = 24.5–103.7 mg/kg dm). This is the case for the *M. muelleri* from NF and NS, except that the Se concentration in the NS samples was rather high (Se_{NS} = 2.48 mg/kg dm).

Compared with other animal food products, the *M. muelleri* from all the sampling areas had higher concentrations of vitamin A₁ and DHA than whole *Sardina pilchardus* (vitamin A₁ = 115 ± 32.7 μ g/100g and DHA = 0.87 ± 0.15 g/100g; Aakre et al., 2020), a higher concentration of Se than whole *Engraulis encrasicolus* (Se = 38.2 ± 2.1 μ g/100g; Aakre et al., 2020), filet of commercial fishes (e.g. Se_{Salmo salar} = 17 and Se_{Gadus morhua} = 25 μ g/100g; Alvheim et al., 2020) and other animal products (e.g. Se_{pork/beef} = 6 and Se_{chicken} = 12 μ g/100g; Alvheim et al., 2020). These comparisons may involve multiple tissue types, and data from whole fish samples may contain greater variability than that from a single tissue type. Such variability is likely due to different accumulation mechanisms among substances in tissues/organs (Landrier et al., 2012; Shukla et al., 2018), and possibly gut contents of individual whole fish. While this variability may have made the comparisons across tissue types less robust, it nevertheless highlights the possible range in the substance yields from the consumer's perspective.

Vitamin D₃ is another important micronutrient (Holick, 2007; Amrein et al., 2020) which can be obtained from seafood (Lund, 2013). In our study, most of the measured vitamin D₃ concentrations were <LOQ (LOQ = 0.01 mg/kg) except in two instances (0.01 [from BB] and 0.02 mg/kg [from NS]; Appendix 1). According to the EU Regulation No 1169/2011, a food item that provides 15% of 5 μ g/100 g vitamin D₃ (or 0.0075 mg/kg in

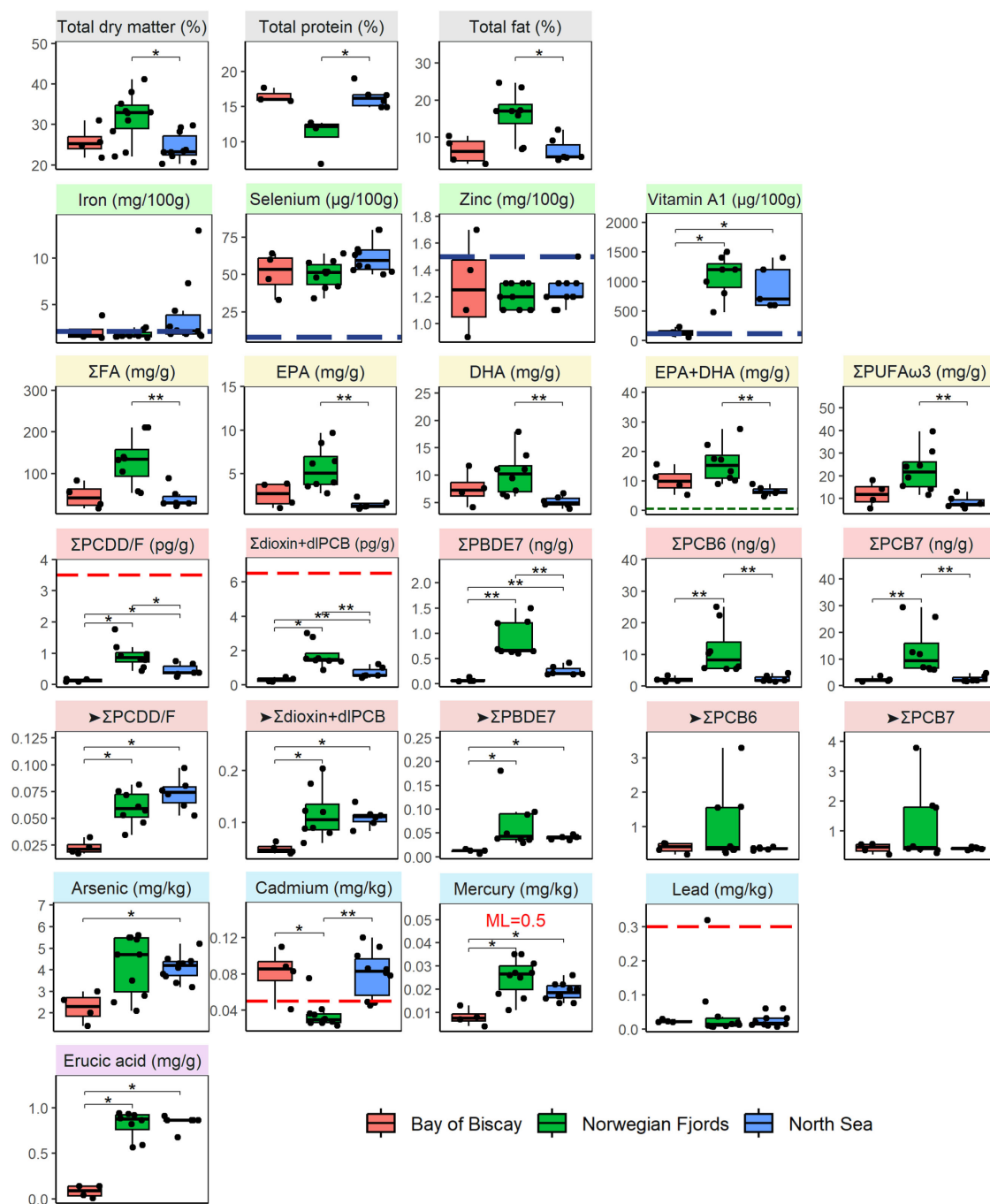


FIGURE 2

Non-parametric comparison of gross proximate (grey), nutrient (micro- and macro-) and undesirable substance concentrations (persistent organic pollutants (red), metals & metalloids (blue), and others (purple)) of the *Maurolicus muelleri* in wet weight from the Bay of Biscay, Norwegian fjords, and the North Sea. Blue long-dashed line indicates the concentration for a significant source of a micronutrient; green dashed line indicates the concentration for a source of omega-3 fatty acids (ω-3 FAs; the lower line) or it contains high ω-3 FAs (the higher line); red long-dashed line indicates the maximum level (ML) for an undesirable substance; for mercury, the ML is annotated. >: the fat-corrected values. Statistical significance (non-significance not shown) using Wilcoxon test: $p_{adjusted} < 0.05$ and $** < 0.01$. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated FA; PCDD/F, total dioxin including PCDD (polychlorinated dibenzodioxin) and PCDF (polychlorinated dibenzofuran); PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; and dl-PCB, dioxin-like PCB.

concentration) can be qualified as a significant source of vitamin D₃. Because the LOQ of our method was higher than the regulation value, we cannot confirm whether the *M. muelleri* collected in this study is a significant source of vitamin D₃.

Malnutrition might be exacerbated due to the pandemic, food shortage (Workie et al., 2020; Zurayk, 2020), and climate change (Gomez-Zavaglia et al., 2020), while the global population is projected to increase. Thus, alternative productive and nutritious foods such as *M. muelleri* are important to help combat malnutrition, for example, reducing the risk of iron deficiency-

induced anaemia (Aikawa et al., 2006; Beck et al., 2014) which is prevalent globally (Stevens et al., 2022). Its high Se concentration may help provide a protective effect against cardiovascular diseases (Mozaffarian, 2009) and Hg poisoning (Gochfeld and Burger, 2021).

Food safety

The *M. muelleri* from all the sampling areas were found to have low risk. All the examined undesirable substances had low

TABLE 4 Summary of standard length and important substance values (mean \pm S.E.; in wet weight) measured for *Maurolicus muelleri* composite samples of whole fish individuals collected from the Bay of Biscay, Norwegian fjords (Bjørnafjorden, Boknafjorden, and Osterfjorden) and the North Sea.

Substance [EU regulation values]	Sampling area		
	Bay of Biscay (n = 4)	Norwegian fjords (n = 10)	North Sea (n = 10)
Standard length (mm)	28.98 \pm 5.78	41.08 \pm 8.91	40.93 \pm 8.62
Gross proximate (%)			
Total dry matter	25.78 \pm 1.92	31.89 \pm 1.90	24.36 \pm 1.10
Total protein	16.50 \pm 0.59 (3)	10.83 \pm 1.39 (4)	16.32 \pm 0.62
Total fat	6.40 \pm 1.80	16.16 \pm 2.03 (8)	6.48 \pm 1.34 (6)
Micronutrients			
Iron [2.1] (mg/100g)	2.13 \pm 0.57	1.78 \pm 0.11	3.84 \pm 1.16
Selenium [8.25] (μ g/100g)	51.00 \pm 7.01	50.10 \pm 2.90	62.10 \pm 3.48
Zinc [1.5] (mg/100g)	1.28 \pm 0.18	1.20 \pm 0.03	1.24 \pm 0.04
Vitamin A ₁ [120] (μ g/100g)	132.50 \pm 37.50	1082.86 \pm 133.84 (7)	900.00 \pm 167.33 (5)
Macronutrients (mg/g)			
		(n = 8)	(n = 6)
Σ FA	45.35 \pm 15.15	130.52 \pm 18.58	41.04 \pm 10.31
EPA	2.59 \pm 0.71	5.62 \pm 0.79	1.44 \pm 0.21
DHA	7.58 \pm 1.57	10.36 \pm 1.25	5.13 \pm 0.41
EPA+DHA [0.4 & 0.8] *	10.17 \pm 2.20	15.98 \pm 2.02	6.57 \pm 0.61
Σ PUFA ω -3	11.84 \pm 2.72	22.46 \pm 2.93	8.28 \pm 1.09
Persistent organic pollutants			
		(n = 8)	(n = 6)
Σ PCDD/F (WHO-TEQ pg/g) [3.5]	0.13 \pm 0.02	0.93 \pm 0.13	0.46 \pm 0.08
	> 0.02 \pm 0.00	> 0.06 \pm 0.01	> 0.07 \pm 0.01
Σ dioxin+dl-PCB (WHO-TEQ pg/g) [6.5]	0.29 \pm 0.06	1.73 \pm 0.23	0.70 \pm 0.13
	> 0.05 \pm 0.01	> 0.12 \pm 0.02	> 0.11 \pm 0.01
Σ PBDE ₇ (ng/g)	0.07 \pm 0.02	0.89 \pm 0.11	0.25 \pm 0.04
	> 0.01 \pm 0.00	> 0.07 \pm 0.02	> 0.04 \pm 0.00
Σ PCB ₆ (ng/g)	2.10 \pm 0.44	11.42 \pm 2.47	2.28 \pm 0.44
	> 0.38 \pm 0.08	> 1.01 \pm 0.38	> 0.36 \pm 0.02
Σ PCB ₇ (ng/g)	2.31 \pm 0.48	13.14 \pm 2.90	2.61 \pm 0.50
	> 0.42 \pm 0.08	> 1.16 \pm 0.44	> 0.41 \pm 0.02

(Continued)

TABLE 4 Continued

Substance [EU regulation values]	Sampling area		
	Bay of Biscay (n = 4)	Norwegian fjords (n = 10)	North Sea (n = 10)
<i>Trace metals (mg/kg)</i>			
Arsenic	2.25 ± 0.35	4.23 ± 0.39	4.09 ± 0.19
Cadmium [0.05]	0.08 ± 0.01	0.03 ± 0.00	0.08 ± 0.01
Mercury [0.5]	0.01 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Lead [0.3]	0.02 ± 0.00	0.05 ± 0.03	0.03 ± 0.01
<i>Other undesirable substance</i>			
Erucic acid (C22:1 ω-9) (mg/g)	0.08 ± 0.03	0.81 ± 0.05 (8)	0.84 ± 0.03 (6)

Relevant EU regulation values for individual substances (if available) are indicated. The guideline values for micronutrients are calculated from nutrient reference values (see methods section for the calculation). Each composite sample contained approximately 25 individual fish. Sample size (n, the number of composite samples) is indicated in bracket for a substance group or an individual substance if differs from that in the table header.

FA, fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated FA; PCDD, polychlorinated dibenzodioxin; PCDF, polychlorinated dibenzofuran; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; and dl-PCB, dioxin-like PCB. >: the fat-corrected values. * EU guideline values for EPA+DHA: 40 mg/100 g (a source of ω-3 FA), and 80 mg/100g (contains high ω-3 FA).

concentrations except for the high Cd concentrations in the BB and NS samples when compared the measured concentrations against the MLs in relevant EU regulations. We further compared the BB and NS Cd concentrations with the EU regulation on undesirable substances in animal feed under EU Directive 2002/32/EC (<https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02002L0032-20191128&from=EN>) and found that they were below the ML (assuming an 88% dry weight). This suggests their potential usage as a feed ingredient, similar to some earlier findings (Berntssen et al., 2021).

The *M. muelleri* from our sampling areas had lower Cd concentrations than those reported from the Mid Atlantic Ridge (Grimaldo et al., 2020), and lower Hg concentrations than those from the Azores (Monteiro et al., 1996).

Compared with other mesopelagic species from BB (Chouvelon et al., 2022) and assuming the dry matter content of *M. muelleri* is 25%, the *M. muelleri* from BB were higher in As concentration than these mesopelagic fishes ($As_{BB} = 90.00$, $As_{Others} = 3.83$ -53.60 mg/kg dm); this is also the case for Cd ($Cd_{BB} = 3.20$, $Cd_{Others} = 0.03$ -2.43 mg/kg dm), and Pb ($Pb_{BB} = 0.80$, $Pb_{Others} = 0.03$ -0.18 mg/kg dm). While the Hg concentrations in the *M. muelleri* from BB were similar to these mesopelagic species ($Hg_{BB} = 0.40$, $Hg_{Others} = 0.090$ -1.365 mg/kg dm). These differences persist when comparing the *M. muelleri* from NF and NS with these mesopelagic species except that the Cd concentration in the NF samples was rather low ($Cd_{NF} = 1.2$ mg/kg dm), and the Hg concentration in the NF samples was rather high ($Hg_{NF} = 1.2$ mg/kg dm).

Compared with other mesopelagic species from NF (Wiech et al., 2020), the *M. muelleri* from all the sampling areas had lower concentrations of As, and Pb than *Meganctiphanes norvegica* ($As = 28 \pm 19$, $Pb = 0.086 \pm 0.075$ mg/kg), As, and Hg than *Pasiphaea* sp. ($As = 22 \pm 19$, $Hg = 0.038 \pm 0.02$ mg/kg), As, and Pb than *Eusergestes arcticus* ($As = 9.5 \pm 4.2$, $Pb = 0.01 \pm 0.006$ mg/kg), and Pb than *Benthosema glaciale* ($Pb = 0.016 \pm 0.017$ mg/kg). The *M. muelleri* from NF and NS had higher concentrations of total fat and erucic acid than the aforementioned mesopelagic species from NF;

while those from BB and NS had lower concentrations of total PCDD/F, PBDE₇, PCB₆, and PCB₇ than these mesopelagic species from NF.

There are other undesirable substances that were not included in the present study. For example, wax esters may pose some health hazards (e.g. keriorrhea) when consumed in large amounts (Schots et al., 2020) and are commonly found among mesopelagic species (Voronin et al., 2022, 2023). However, the wax ester concentrations in the NF and NS samples were low (in both wet weight and % of FA; Appendix 1), which is similar to some of the previous findings (Wiech et al., 2020; Voronin et al., 2023).

Observed differences and possible drivers

Some of the analysed substances had great variation across the sampling areas, and there are multiple possible drivers behind them. The differences in the gross proximate across the sampling areas indicate differential accumulation mechanisms for the *M. muelleri*. Evidently, fishes from northern regions and/or collected during colder seasons tended to have larger lipid reserves (Schultz and Conover, 1997) and unsaturated FAs concentrations (Hazel, 1984) than those from the south or collected during warmer seasons. This matches with our data that the samples from NF (colder) had higher fat content than the samples from NS & BB samples (warmer). Additionally, smaller-body sized fishes often have lower lipid reserves than larger-sized ones (Schultz and Conover, 1997; Toppe et al., 2007); this has been shown among *M. muelleri* (Olsen et al., 2020). In our study, such a body size-fat correlation was apparent between the BB and NF samples but not between the NF and NS samples (Table 4). The body sizes of the NF and NS samples were similar, yet the fat content of the NF samples was statistically significantly higher than that of the NS samples (Table 4). This was likely caused by the sampling during different months/seasons (NF: March, May, and December; NS: March) and high food availability in the fjords due to the seasonal algal blooming (Cembella et al., 2005; Marquardt et al., 2016). Moreover, biological activities such as spawning can lead to

transferring essential nutrients to the eggs and metabolising the stored fat for reproduction, thus resulting in the loss of some substances in a short period (Boran and Karaçam, 2011; Fuiman and Faulk, 2013; Shadyeva et al., 2019). Although *M. muelleri* has a protracted spawning season (Gjøsæter, 1981; d'Elbée et al., 2009), the samples collected in BB were practically outside of the spawning season (spawning season: at least between March and September; Alvarez et al., 2023), while those in NF and NS partially or entirely included spawning individuals (spawning season: March to September; Gjøsæter, 1981). Thus, we could not examine the effect of spawning here. Lastly, *M. muelleri* can have complex population structures (Ikeda, 1994; Rasmussen et al., 2009) with individuals of different life stages occurring in the same location, potentially resulting in variability in the substance concentrations.

For the macronutrients, the trend in fat content (i.e. NF > NS [statistically significant] and NF > BB [marginal]) was also observed for total FAs and individual FAs/FA groups, possibly for similar reasons such as the lower temperature and higher food availability due to the seasonal algal blooming in NF than in BB and NS (e.g. Donnelly et al., 1990; Zlatanov and Laskaridis, 2007; Boran and Karaçam, 2011). Among the micronutrients, the only observed difference across the sampling areas was the lower concentration of vitamin A₁ in the BB samples than in the NF and NS samples. This was likely due to the seasonal variation in vitamin A₁ which has been observed among a few other fish species (Bridges, 1965; Temple et al., 2006). Since our knowledge of vitamin A₁ is rather limited, we cannot disentangle the temporal effects from the spatial effects based on our data.

The POP concentrations (in wet weight) showed similar variation patterns across the sampling areas to those of fat/FAs that higher POP concentrations were found in the NF samples than in the BB and NS samples. Such a co-occurrence of fat/FAs and POPs has been observed in other aquatic systems including the Mediterranean pelagic fishes (Cembella et al., 2005; Romanić et al., 2021) and the commercial fishes from the North East Atlantic Ocean (Ho et al., 2021). This co-occurrence likely results from the interaction between POPs and lipids (Elskus et al., 2005) that POPs often accumulate in fatty tissues and their bioavailability may be affected by the lipid composition. In contrast, the fat-corrected POP levels showed different patterns across the sampling areas compared with those from the uncorrected concentrations. The statistically significant differences in POP concentrations between the NF and NS samples were attenuated, and no statistically significant difference across the sampling areas remained in PCB₆ and PCB₇ concentrations. Because the samples were collected during different seasons, the anticipated variation in fat content across the sampling areas may drive the variation in POP concentrations to some extent. In addition, POP concentrations can be different at the base of the food web geographically across the large sampling areas (Batool et al., 2016), resulting from the grasshopper effect of POPs (Fernández and Grimalt, 2003). This may have led to higher basal POP in NF and NS than in BB. Besides, the samples collected from one of the selected fjords, Osterfjorden (OF), had higher PBDE₇, PCB₆, and PCB₇ concentrations and the pertinent fat-corrected levels than those from the other two fjords; this has also been observed among a few other fish species (Frantzen and Måge, 2016).

Because the fat content of the OF samples were lower than those from the other two fjords (Appendix 3), the POP concentrations in OF may not be fat-driven. We suspect that such an observation may result from the high basal PBDE₇, PCB₆, and PCB₇ concentrations in OF. Overall, all the measured POP concentrations were statistically significantly lower than the MLs, thus, the sampled *M. muelleri* had low risks of POPs to human health.

The samples from both NF and NS had higher Hg concentrations than those from BB. However, this trend is opposite from what has been observed in different fish species from the south to the north in the North East Atlantic Ocean (Azad et al., 2019). Because Hg concentration correlates positively with body size (Monteiro et al., 1996), our contradictory result suggests that body size-dependent bioaccumulation may play a predominant role here as the NF and NS samples had greater body size than the BB samples (Table 4). On the other hand, the NS samples had Hg concentrations marginally lower than the NF samples while both had similar body sizes (Figure 2). This is in accordance with earlier findings in several other fish species collected from NS and NF (Azad et al., 2019).

The As concentrations, however, were lower in BB than in NF and NS. It may likely result from different temperatures, which has been observed in As concentrations globally (higher in polar regions than in tropical regions; Fattorini et al., 2006), as well as the body size-dependent bioaccumulation of As (Zhang et al., 2022). Additionally, the higher As concentrations in the NF samples than the NS samples may result from different temperatures or fat contents during the sampling seasons (i.e. March, May, and December vs. May, respectively; Appendix 2). There is no authorised ML for arsenic in European legislation. To assess the potential risk from As, the toxic forms must be considered. Earlier work has shown that only a small fraction of the total arsenic in *M. muelleri* was present in the most toxic inorganic form (Wiech et al., 2020). And a recent study performing As speciation found large proportions of potentially toxic arseno-lipids (Tibon et al., 2022). But as the toxicity of arseno-lipids depends on the present As species, a complete analytical characterisation of present compounds is needed to evaluate the risk connected to arseno-lipids.

The higher Cd concentrations in the *M. muelleri* from BB and NS than those from NF are consistent with earlier findings between NF and offshore systems (Wiech et al., 2020) and with another common mesopelagic species *B. glaciale* (Bjordal and Thorvaldsen, 2020; Wiech et al., 2020). One possible driver may be the Cd distribution in seawater. Cadmium is known to behave similarly to phosphate (de Baar et al., 1994), that it is depleted close to the surface (where primary production takes place) and enriched in deeper waters (where organic matter is decomposed). Thus, higher concentrations of Cd are expected in BB and NS which receive water from oceanic deep-water systems than in NF which receive a mixture of oceanic water and fresh water.

Most of the examined undesirable substances had concentrations lower than the relevant MLs, yet some had great variation across the sampling areas. Also, POPs may have a cumulative adverse effect on human health (Bank et al., 2020) and other undesirable substances like Hg may mask the positive

effects of essential nutrients on human health (Kris-Etherton et al., 2002). Thus, it is crucial to monitor not only the mean values but also the variation in concentrations to further influence their usage (food or feed). One possible way to further reduce the POP concentrations is to process the fish for fish oil and fish meals through the established methods (Berntssen et al., 2021).

Other drivers

Both biological and environmental drivers are important in determining the substance concentrations of the *M. muelleri* in the present study. However, many drivers from these two dimensions are entangled and may synergistically affect the substance concentrations in fish. Apart from the discussed drivers, there are others that may incorporate both dimensions. For example, temporospatial variation in food availability (e.g. algal blooming in NF) can affect the substance concentrations in fish (Boran and Karaçam, 2011) as well as their feeding strategies. Since much of the substances in fish come from their diet (Grimaldo et al., 2020), apart from their geographic distribution (Batool et al., 2016), mesopelagic food webs may be supported by multiple food sources (Ianiri and McCarthy, 2023) with different basal substance compositions (e.g. upwelling, offshore and nearshore phytoplankton, and terrestrial runoff). While the prey specialisation is relatively low among mesopelagic fishes (Bernal et al., 2015), the substance concentrations in fish are thus partially determined by the contributions of prey from different basal food sources. Thus, it is important to understand the nutrient flow in mesopelagic species, which may nevertheless provide insights into the possible environmental impacts and sustainability of potential fisheries. For mesopelagic systems, these are particularly vital (Hidalgo and Browman, 2019; Grimaldo et al., 2020; Standal and Grimaldo, 2021; Fjeld et al., 2023) due to their trophic functions in nutrient cycling, carbon fixation (Li et al., 2022; Schadeberg et al., 2023), and mediating ocean health (Bank et al., 2020).

Limitations and future work

In this study, we chose nutrients and undesirable substances based on relevant studies and EU regulations. However, there is no EU regulation on a few important undesirable substances including As, wax esters, and PBDE. As they may pose threats to human health, it is important to develop regulations on them to further ensure food safety of current and emerging aquatic foods.

We mentioned the importance of body size on the concentrations of some nutrients and undesirable substances. However, in the present study, only the body size of a group of individuals for each catch was measured, which reduced the resolution in variability to understand the effect of body size on the substance concentrations. It is ideal to analyse the substance concentrations at the individual level. Unfortunately, the current methods require a higher mass of materials than one individual of a small fish such as *M. muelleri* to analyse the full suite of nutrients

and undesirable substances. Thus, future development of analytical methods is essential to enable high-resolution individual-based substance analyses of small mesopelagic fishes.

Nevertheless, we suggested that the season of collection and spawning activities may have caused some uncertainty in the analyses. The sampling of mesopelagic species at IMR and AZTI takes into consideration of the cruise planning and financial feasibility. Targeted sampling of mesopelagic species is challenging, and cruise activity was limited. This led to sampling at different seasons in different areas and varying size distributions. As *M. muelleri* is known to spawn in batches over a protracted spawning period and shows mixed schooling behaviours, it was not possible to foresee their maturity levels prior to sampling. These factors may have an influence on the substance concentrations. However, our datasets nevertheless have important implications on the geographic differences in the substance concentrations of the *M. muelleri* in the North Atlantic Ocean, while pointing to important future work to better inform the sampling design and improve the resolution of such analyses by disentangling these potentially confounding factors.

Conclusion

We confirmed the potential roles of *Maurollicus muelleri* in food security and nutrition. There are statistically significant differences in the concentrations of several substances across the sampling areas, including vitamin A₁, dioxin, cadmium, and mercury. These differences were probably related to differential basal substance concentrations, body sizes/maturity levels of samples, and sampling periods. One general trend we found is that the *M. muelleri* from Norwegian fjords tended to contain higher concentrations of fat, fatty acids, and lipophilic undesirable substances than those from the Bay of Biscay and the North Sea, however, the drivers behind are unclear. Overall, the *M. muelleri* from all the sampling areas are good sources of several essential nutrients (e.g. Se and vitamin A₁) with low concentrations of undesirable substances (e.g. mercury) according to available EU regulations for food. However, the variability in some of these substances (e.g. cadmium) should be closely monitored to provide important indications on the usages of this marine resource (i.e. for food or feed).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the animal study because We do not deal with live animals in this study. All the animal data we used here was from previous studies/projects.

Author contributions

YZ, AA, LM, and MW contributed to the conception and design of the work. YZ, MK, CB, BI, PA, GB, MB, and MW contributed to the sample collection and data analysis. YZ and MW contributed to the drafting the work. All authors contributed to the revising the work and approved for publication of the content. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1213612/full#supplementary-material>

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