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Lipids in meso- and bathypelagic fishes from the North Atlantic Ocean: dietary inputs suggested from fatty acid trophic markers

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ABSTRACT: Trophic interactions of mesopelagic fishes are key pathways in the vertical transport of carbon through the biological carbon pump. However, diet and feeding behaviours of many members of the mesopelagic community, including lanternfish (Myctophidae) and bristlemouths (Gonostomatidae), remain poorly resolved. We obtained specimens from 1 species of lanternfish, *Benthosema glaciale*, and several bristlemouths of the genus *Cyclothone*, from 8 locations in the Northeast Atlantic Ocean, within 19–22° W and 20–55° N, at depths down to 1900 m, and used fatty acid trophic marker (FATM) analysis to assess the main dietary sources and potential feeding habits of those fish. We compared the FA profiles of the fish to those of their zooplankton prey sampled at the same time and locations. The fatty acid composition of the fish separated them into 3 distinct groups. The first group, which included only *B. glaciale*, was characterized by the phytoplankton trophic markers 16:4 and 18:4(n-3), indicating that the fish had fed on primary consumers, likely zooplankton. The second group included *C. microdon* and 2 unidentified *Cyclothone* sp. and was characterized by the monounsaturated FA markers 20:1(n-9) and 22:1(n-11), indicative of lipid-rich calanoid copepods. Finally, the third group, comprising *C. pseudopallida* and 22 unidentified *Cyclothone* sp., was characterized by bacterial FA markers (15:0 and 17:0) and saturated FAs (16:0 and 18:0) that, in the deep sea, are likely associated with marine snow. The results show that meso- and bathypelagic fishes sampled at different locations will display differences in FA profiles, reflecting distinct dietary sources. In this way, FATMs may facilitate a better understanding of trophic interactions and energy transfer in deep ocean ecosystems.

KEY WORDS: Mesopelagic · Trophic interactions · Lanternfish · Myctophidae · Bristlemouths · Gonostomatidae · *Cyclothone* · Diet · Feeding habit

1. INTRODUCTION

The meso- and bathypelagic zones are arguably the largest ecosystems on the planet and among the least studied portions of the world ocean (St. John et al. 2016). They provide habitats for diverse fish communities (Gjøsaeter & Kawaguchi 1980). Lanternfishes (Family Myctophidae) are characteristic of those communities and contribute the highest species diversity (Gjøsaeter & Kawaguchi 1980, Hulley 1981, Gibbs & Krueger 1987), while the bristlemouth genus *Cyclothone* (Family Gonostomatidae) is likely the most abundant of all vertebrate genera (Thompson & Kenchington 2017). Collectively, meso- and bathypelagic fishes are certainly the most numerous vertebrates in the biosphere (Nevenzel & Menon 1980, Nelson 2006).

It is challenging to make reasonable estimates of the global biomass of mesopelagic fishes and even

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more so, of bathypelagic fishes. Current estimates range from 10^9 t (Gjøsaeter & Kawaguchi 1980, Bar-On et al. 2018), which corresponds to the total biomass of all other terrestrial and marine vertebrates combined (Bar-On et al. 2018), to an order of magnitude higher (10^{10} t: Irigoien et al. 2014). Clearly, investigation of the functioning of such massive biomass in the vast meso- and bathypelagic ecosystems is needed to understand the impact of the fish on global oceanic carbon budgets, as well as their role in carbon transfer and cycling.

The main carbon transport by meso- and bathypelagic fishes is through active diel vertical migration (DVM) (Pinti et al. 2023). Many species of lanternfishes undertake DVM, spending the night foraging on zooplankton in surface waters (Merrett & Roe 1974, Gartner et al. 1987) and returning to the safety of the mesopelagic zone at dawn where they can avoid visual predation (Suntsov & Brodeur 2008, Mehner & Kasprzak 2011, Sutton 2013). At depth, the diel vertical migrants excrete and respire the organic carbon ingested at the surface (Olivar et al. 2019). Mesopelagic fishes alone may facilitate up to 40% of the deep carbon flux through their vertical migration (Davison et al. 2013, Trueman et al. 2014, Pinti et al. 2023), making them particularly important for global carbon sequestration. While DVM is characteristic of the mesopelagic fish community, the migration pattern varies among species, and some remain at depth, relying rather on the migration of their prey or chance encounters to provide opportunities to feed (Watanabe et al. 1999).

Considering their large biomass, meso- and bathypelagic fishes are both major consumers as well as important prey within marine food webs. They are key prey items for economically important species including tuna, squid and sharks, as well as other charismatic animals such as manta rays, penguins and other seabirds, and marine mammals (Potier et al. 2007, Spitz et al. 2010, Watanuki & Thiebot 2018). As consumers, meso- and bathypelagic fishes span from zooplanktivorous to carnivorous (Papadimitraki et al. 2023). Moreover, most fishes are opportunistic feeders on a diverse range of different zooplankton prey, such as different species of zooplankton (e.g. copepods, euphausiids, amphipods), as well as fish eggs and larvae, while others are partially or fully piscivorous (Bernal et al. 2015). However, due to the challenges in investigating deep-water fishes living in and below the mesopelagic zone (Saint-Béat et al. 2015, Silva et al. 2022), knowledge of their feeding ecology remains sparse.

An insight into the feeding history, and to some extent, the feeding mode, of organisms can be attained by investigating their fatty acid (FA) compositions. FA analysis provides a long-term feeding history, as FAs are laid down in the tissues in an integrative manner and, therefore, indicate general trends in the diet. Some dietary sources that cannot be visually identified from gut contents, such as bacteria or marine snow, can be detected by their specific FA trophic markers (FATMs: Dalsgaard et al. 2003).

FATMs are specific FAs and FA ratios that are frequently used to determine the trophic status of marine organisms (Dalsgaard et al. 2003). Unique markers can be traced, often unmodified, from primary producers into the membranes and reserves of consumers, providing important dietary information on their previous (weeks to months) feeding history (Graeve et al. 1994, Galloway & Budge 2020). For example, first-order consumers, such as herbivorous zooplankton, are typically characterized by high contents of diatom markers, such as 16:1(n-7) and 16 polyunsaturated FAs (PUFAs) (Jónasdóttir 2019). FATMs can be traced into second-order consumers, providing information about their dietary sources (St. John & Lund 1996). Long-chain monounsaturated FAs (MUFAs) and fatty alcohols (FAlcs), such as 20:1(n-9) and 22:1(n-11), are characteristic of lipid-rich copepods of the genus *Calanus* (Sargent & Falk-Petersen 1981, Kattner & Hagen 1995). The odd-chain FAs, including 15:0 and 17:0, are characteristic of bacteria (Perry et al. 1979). Elevated levels of saturated FAs (SFAs), such as 16:0, 18:0 and 22:0, are indicative of detrital feeding, as PUFAs are scarce in detritus (Zhukova 2019). When the FA 18:1(n-9) is found in a high proportion in fish, it can be an indication of carnivory (Sargent & Falk-Petersen 1981, Falk-Petersen et al. 2000). Specifically, most organisms can produce 18:1(n-9), while 18:1(n-7) is of phytoplankton or bacterial origin, elongated from 16:1(n-7). Therefore, high values of the ratio of 18:1(n-9)/18:1(n-7) are considered indicative of carnivory (Hagen et al. 1995, Cripps et al. 1999). Another marker for carnivory is the FA 22:6(n-3), docosahexaenoic acid (DHA). DHA is one of the main building blocks of membranes, and it is specifically retained and accumulated by organisms, suggesting carnivory (Stevens et al. 2004).

Atlantic meso- and bathypelagic fishes were sampled during a multidisciplinary bathypelagic cruise on RV 'Sarmiento de Gamboa'. The aim of the expedition was to assess the different fluxes involved in ocean carbon sequestration by evaluating migrant

biomass, gut, respiratory, excretory, moulting and lipid fluxes in the Atlantic bathypelagic zone. We used FATMs to explore the feeding ecology of several fishes, with the main objective of gaining insight into their diet and feeding history. Specifically, we assessed the FA composition of specimens of the lanternfish *Benthosema glaciale*, which is a characteristic diel vertical migrant of the North Atlantic, the bathypelagic non-migrating bristlemouth *Cyclothone microdon* and, for the first time, we present the full FA profile of the mesopelagic *C. pseudopallida*. We show that meso- and bathypelagic fishes sampled at different locations will display differences in FA profiles, reflecting distinct dietary sources, and we conclude that the relative abundances of characteristic FATMs observed in such fishes can indicate different pathways of carbon sources from lower trophic levels.

2. MATERIALS AND METHODS

2.1. Sampling

Meso- and bathypelagic fishes (N = 36) were opportunistically collected in the northeast Atlantic in May and June 2018 during the 'Bathypelagic' expedition of the RV 'Sarmiento de Gamboa', operated by the Spanish National Research Council (CSIC). Copepods, as known prey of these fishes, were also captured during the expedition. Samples were gathered along a 10-station transect that followed longitude 20°W from 20 to 55°N (Fig. 1). At each station, meso- and bathypelagic fauna, including fishes and micronekton, were collected during both daylight and night using a Mesopelagos 7-net opening/closing system, with a mouth opening of 35 m^2, a total length of 50 m and graded meshes in each net starting with 30 mm and terminating at 4 mm (Meillat 2012). The discrete layers sampled by the Mesopelagos net differed among the stations, and the depths of capture for the analysed specimens are presented in Table 1 and in Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m717p127_supp.pdf. Each haul took approximately 8 h. Echograms were recorded during the cruise and are presented separately by Peña et al. (2020).

Most of the meso- and bathypelagic fishes collected belonged to either Myctophidae or Gonostomidae. These were identified to the lowest taxonomic level possible but, because of the fragility of these fishes, some individuals, including 14 specimens of *Cyclothone* spp., could not be identified to species. The selected fish specimens were immediately placed in vials or sealed zip-lock bags under a nitrogen atmosphere to prevent oxidation of lipids and stored at −80°C until further analysis.

Zooplankton, including copepods, were sampled using a Multiple Opening/Closing Net and Environmental Sensing System (MOCNESS) fitted with 200 µm mesh nets (n = 8). At each station, the MOCNESS was deployed to 1900 m depth, where the first 5 nets sampled 300 m depth intervals, nets being exchanged at 1600, 1300, 1000, 700 and 400 m depth. The remaining 3 nets sampled 400–200, 200–100 and 100–0 m layers. After retrieval, copepods captured by each of the nets were sorted into species and 1–5 individuals of each species were placed in cryovials, topped with nitrogen and immediately frozen at −80°C. Samples were processed to analyse their FA content and composition from different depths

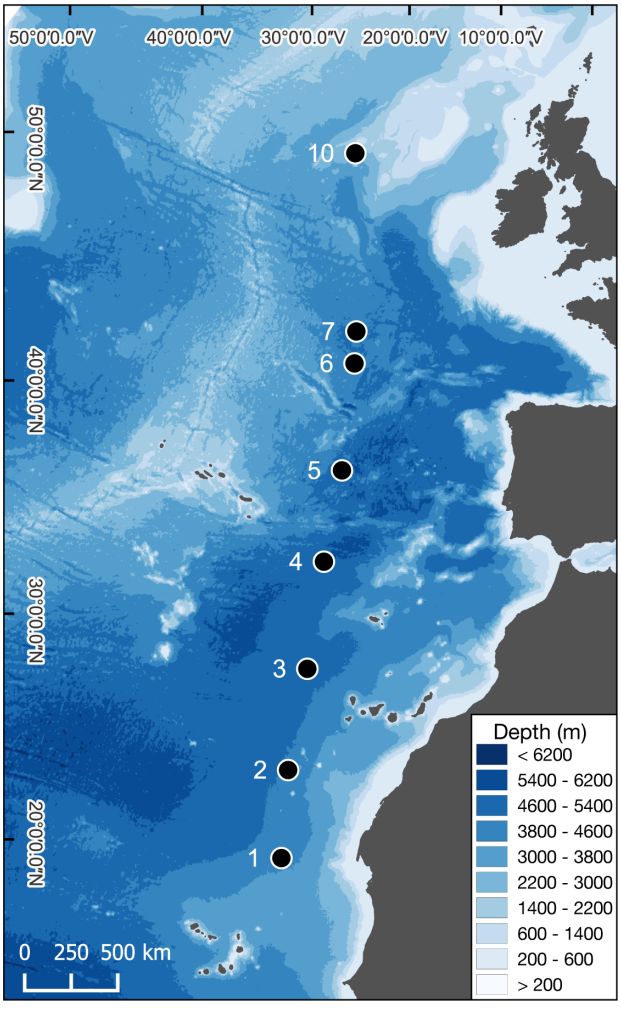

Fig. 1. Stations sampled for meso- and bathypelagic fishes and copepods by RV 'Sarmiento de Gamboa' during May 2018. Fish were sampled at all stations, while copepods were sampled at all stations except Stn 3

Table 1. Summary of meso- and bathypelagic fishes analyzed (N: number of specimens; stations of capture coded as ST-D: daylight haul, ST-N: night haul). Standard length, wet weight (ww) and lipid content shown as means ± SD. Bottom: Mean values for the 4 taxa into which the specimens were classified

Species	N	Station	Depth (m)	Date	Latitude (° N)	Longitude (° W)	Standard length (mm)	Wet weight (g)	Lipid content (% ww)
Cyclothone sp.	1	ST1-D	400–700	28.05.2018	20° 41.58	21° 02.05	28	0.09	1.0
Cyclothone sp.	1	ST1-D	1600–1900	28.05.2018	20° 41.58	21° 02.05	37	0.20	1.2
Cyclothone sp.	3	ST1-N	0–200	29.05.2018	20° 54.87	21° 06.51	48 ± 5	0.44 ± 0.06	0.7 ± 0.1
C. pseudopallida	2	ST2-D	200–500	31.05.2018	25° 00.37	21° 03.93	52 ± 1	0.33 ± 0.16	0.9 ± 0.0
C. pseudopallida	2	ST2-N	800–1200	31.05.2018	24° 55.72	21° 06.55	44 ± 9	0.50 ± 0.01	1.0 ± 0.1
Cyclothone sp.	3	ST2-N	0–200	31.05.2018	24° 55.72	21° 06.55	54 ± 5	0.49 ± 0.14	1.4 ± 0.5
Cyclothone sp.	2	ST3-N	400–700	03.06.2018	30° 01.57	19° 59.58	26 ± 4	0.06 ± 0.04	4.4 ± 1.5
Cyclothone sp.	2	ST4-D	1200–1700	07.06.2018	35° 20.05	20° 16.07	43 ± 3	0.28 ± 0.09	1.2 ± 0.1
Cyclothone sp.	2	ST4-N	400–800	07.06.2018	35° 13.19	20° 00.71	53 ± 1	0.53 ± 0.05	5.1 ± 0.5
C. microdon	2	ST5-N	1200–1700	09.06.2018	39° 53.36	19° 49.59	42 ± 0	0.30 ± 0.01	7.6 ± 4.3
C. microdon	3	ST6-D	0–100	13.06.2018	45° 10.89	19° 55.12	52 ± 7	0.42 ± 0.12	4.1 ± 0.9
C. microdon	1	ST6-D	800–1200	13.06.2018	45° 10.89	19° 55.12	42	0.38	4.5
Benthosema glaciale	7	ST7-N	0–100	14.06.2018	46° 44.99	20° 06.03	36 ± 2	0.47 ± 0.09	3.1 ± 0.3
C. microdon	3	ST10-D	0–1700	10.06.2018	55° 22.42	22° 30.44	57 ± 2	0.78 ± 0.16	3.7 ± 1.5
C. microdon	2	ST10-N	1200–1500	19.06.2018	55° 22.42	22° 30.44	52 ± 1	0.46 ± 0.02	6.4 ± 0.0
C. pseudopallida	4		675				47 ± 7	0.42 ± 0.14	1.1 ± 0.2
C. microdon	11		938				50 ± 7	0.47 ± 0.22	4.6 ± 2.5
Cyclothone spp.	14		433				44 ± 12	0.36 ± 10.21	1.7 ± 1.6
B. glaciale	7		50				36 ± 2	0.47 ± 0.09	3.1 ± 0.3

and locations. We subsequently selected 26 samples of copepods for lipid analysis, including examples of 18 species (some from more than 1 location), which represented the copepods available at the locations and depths from which the mesopelagic fish were collected.

2.2. Lipid analysis

Fishes were kept on dry ice until analysis, as well as between all steps leading to the lipid extraction. The samples were thawed, weighed, measured for standard length and photographed. Each fish was subsequently placed in an individual glass vial, and the lipids were extracted in a 2:1 v/v chloroform:methanol solvent system (Folch et al. 1957). The time between thawing and extraction was well under 5 min. Due to the small size of the fish (<0.8 g), we used whole individuals for the extraction. Internal FAlc (19:0) and FA (23:0) standards were added into each vial before the fish was homogenized for 1 min with an Ultra Turrax™ T18 disperser at 4500 × *g*. The homogenate was subsequently filtered through a phase-separating (grade 902) filter paper. The solvent phase was drawn off into pre-weighed vials and evaporated under nitrogen. The lipids were weighed to the nearest µg before trans-esterification in methanol containing 1.5% sulphuric acid at 40°C overnight, to generate FA methyl esters (FAMEs) (Folch et al. 1957). The FAMEs were purified and separated using thin-layer chromatography (TLC) using a hexane:diethyl ether:acetic acid (90:10:1, v/v/v) solvent system. The FAMEs were isolated from the plates and extracted using a 1:1 hexane:diethyl ether solvent system which was evaporated before adding hexane to obtain 1 mg ml^{-1} stock solutions. The FAME samples were analysed using a Thermo Finnigan gas chromatograph with flame ionization detector (GC-FID) with a ZB wax 0.25 mm × 30 m column (Phenomenex). Copepod samples were processed using the same method except that they were not homogenized but sonicated for 5 min in an ultrasonic bath.

FAlcs were isolated from the same TLC plates using the previously described solvent system. FAlc bands were scraped, and the alcohols eluted using 3 ml of 1:1 hexane:diethyl ether, with the addition of 2 ml of $NaHCO_3$ solution. The upper organic phase was removed and placed into clean glass vials, with the solvent dried under nitrogen. We added 100 µl of BSTFA + TMCS (1%) and 50 µl of pyridine to the samples, which were then incubated at 50°C for 1 h. Samples were once again dried under nitrogen and re-suspended in 25 or 100 µl of hexane, depending on alcohol content, prior to analysis on a gas chromatograph-mass spectrometer (GC-MS), with 1 µl

injected. Alcohol analysis was run on a Thermo Polaris Q using an HP-5 MS UI (30 m × 0.25 mm, 0.25 µm) column (Agilent).

2.3. Statistical analysis

The FA and FAlc profiles were ordinated by log-ratio analysis (LRA), which is principal component analysis of data transformed to logarithms of pairwise ratios (Aitchison 1990, Greenacre 2018, 2021, Graeve & Greenacre 2020, Greenacre et al. 2022). The method does not allow zero values, hence any measurements below the detection limit of the FA analysis must be replaced with an appropriate, small positive value. As zeros in the data were not structural but reflected the limit of detection of the chromatograph, we replaced them with half the minimum detected value for each FA and subsequently renormalized the dataset. No weights were assigned to the FAs and therefore the components of the LRA were treated equally.

We tested overall group differences using a non-parametric permutation test performed using the R package 'vegan' (Oksanen et al. 2020) with 99 999 permutations. We computed 95% confidence regions for the bivariate means of groups of specimens based on bootstrapping (Greenacre 2016). The statistical analyses and plotting were performed using the 'easyCODA' package (Greenacre 2018) in R version 4.0.2. Comparison of FATMs between fish groups was tested by 1-way ANOVA using the 'Sigma Stat' option in Sigma Plot version 13. If the data failed homogeneity of variance or normality tests, they were log-transformed before ANOVA. Pairwise differences were tested by Holm-Sidak all-pairwise multiple comparisons of means.

3. RESULTS

Table 1, Fig. 1 and Fig. S1 summarize the capture locations and biometrics of the specimens analysed. The fish belonged to 2 families, Myctophidae and Gonostomatidae. Most were identified to 1 of 3 species: *Benthosema glaciale* (N = 7), *Cyclothone pseudopallida* (N = 4) or *C. microdon* (N = 11). The remaining 14 specimens were only identifiable to genus because of damage during capture, but each was a *Cyclothone* sp. The *C. pseudopallida* specimens were caught at Stn 2, at the southern end of the survey line (25° N), while *C. microdon* were caught at Stns 5–10. The incompletely identified *Cyclothone* specimens were collected at the southern Stns 1–4. *B. glaciale* were caught at Stn 7 near the surface at night.

3.1. Fatty acid and fatty alcohol composition

The total lipid content of the fishes ranged from 0.7 to 7.6% wet weight (ww) (Table 1) with averages of 1, 4 and 3%ww for *C. pseudopallida*, *C. microdon* and *B. glaciale*, respectively. Unidentified *Cyclothone* types A and B (presented together in Table 1) had 1 and 2% ww, respectively. Mean FA profiles for each species or other group are presented in Table 2, whereas Figs. S2 & S3 provide the compositions of individual specimens. Each group exhibited a high proportion of PUFAs, with mean percentages of 30, 34, 36 and 39% of total FAs (TFAs) for specimens of *C. microdon, Cyclothone* sp., *C. pseudopallida* and *B. glaciale*, respectively.

Whether based on FA or FAlc composition, LRA nested 2 unidentified *Cyclothone* specimens, both of which were taken at Stn 4, amongst the identified *C. microdon* and the other 12 specimens with the identified *C. pseudopallida* (Fig. 2). We categorized the former 2 as *Cyclothone* type 'A' (*C.*A) and the other 12 as *Cyclothone* type 'B' (*C.*B). Specimens of *C. microdon* and those assigned to *C.*A had the highest proportion of MUFAs (59% of TFAs), particularly 18:1(n-9), 18:1(n-7), 22:1(n-11) and 20:1(n-9) FAs. The specimens of *C. pseudopallida* and *C.*B had the highest proportion of SFAs (25–30% of TFAs; Fig. S2), mainly high and relatively high proportions, respectively, of 16:0 and 18:0 FAs.

The PUFA content differed less among the groups. All groups had >6% of 20:5(n-3) (eicosapentaenoic acid, EPA), with *B. glaciale* specimens containing the highest proportion (12% of TFAs). *C. pseudopallida* specimens had the highest proportion, 21%, of DHA, compared to the remaining groups, which presented between 15 and 19% of their TFAs as DHA. All 3 groups had >26% of their FAs as n-3, and more than 16% of the PUFAs had carbon-chain lengths of 22 carbons, meaning that they were highly unsaturated. The *C. pseudopallida* and *C.*B specimens retained small amounts of n-6 PUFAs, mainly 20:4(n-6) and 22:5(n-6), but significantly more than the other groups (Holm-Sidak pairwise comparison between fish groups; $t = 3.1–4.1$ $p = 0.003–0.035$).

The FATMs differed significantly ($p < 0.001$) among the 5 groups of specimens, although pairwise comparisons found no differences between *C. microdon* and *C.*A, nor between *C. pseudopallida* and *C.*B (Table 3). Although *C. microdon* specimens were

Table 2. Percentage composition of fatty acids and fatty alcohols (mean ± SD) in the 5 groups of meso- and bathypelagic fish. *C.*A: *Cyclothone* type A; *C.*B: *Cyclothone* type B; N: number of fish analyzed; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

Fatty acids	*Benthosema glaciale* N = 6	*Cyclothone microdon* N = 11	*C. pseudopallida* N = 4	*C.*A N = 2	*C.*B N = 12	*Cyclothone* spp. N = 14
14:0	3.1 ± 0.4	1.5 ± 0.5	2.2 ± 0.6	1.3 ± 0.5	2.3 ± 0.6	2.1 ± 0.7
15:0	0.2 ± 0.0	0.3 ± 0.2	0.7 ± 0.2	0.1 ± 0.0	0.8 ± 0.2	0.7 ± 0.3
16:0	6.5 ± 0.3	7.0 ± 5.4	18.5 ± 0.9	5.8 ± 0.1	17.6 ± 2.6	15.5 ± 5.2
17:0	0.5 ± 0.2	0.5 ± 0.2	1.0 ± 0.1	0.4 ± 0.2	0.8 ± 0.3	0.6 ± 0.3
18:0	1.9 ± 0.2	1.5 ± 1.3	4.4 ± 0.8	1.0 ± 0.0	3.8 ± 0.9	3.2 ± 1.3
20:0	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
22:0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
24:0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.0 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
SFA total	**12.6 ± 0.6**	**11.1 ± 7.7**	**27.2 ± 1.6**	**8.8 ± 0.1**	**25.9 ± 0.1**	**22.7 ± 7.1**
16:1 (n-9)	0.3 ± 0.0	0.2 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	1.0 ± 1.7
16:1 (n-7)	9.9 ± 0.9	8.1 ± 1.1	6.0 ± 1.3	9.6 ± 0.2	6.5 ± 1.6	6.3 ± 3.2
18:1 (n-9)	29.6 ± 7.2	31.6 ± 6.3	22.4 ± 3.9	25.7 ± 0.2	23.3 ± 2.6	23.8 ± 2.7
18:1 (n-7)	1.9 ± 0.2	4.0 ± 1.0	2.4 ± 0.1	4.3 ± 0.2	2.5 ± 0.3	2.8 ± 0.8
20:1 (n-11)	0.5 ± 0.1	0.9 ± 0.4	0.6 ± 0.3	0.7 ± 1.1	0.6 ± 0.2	0.7 ± 0.4
20:1 (n-9)	1.8 ± 0.1	5.8 ± 3.5	1.4 ± 1.1	10.5 ± 0.6	1.9 ± 1.2	3.5 ± 3.5
20:1 (n-7)	0.2 ± 0.0	0.5 ± 0.2	0.2 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	0.3 ± 0.2
22:1 (n-11)	1.6 ± 0.5	5.0 ± 2.6	1.7 ± 1.7	7.8 ± 0.0	2.4 ± 1.4	3.4 ± 2.5
22:1 (n-9)	0.7 ± 0.2	1.2 ± 0.1	0.4 ± 0.1	1.6 ± 0.3	0.6 ± 0.2	0.7 ± 0.5
24:1 (n-9)	2.5 ± 0.5	1.3 ± 0.2	1.2 ± 0.1	1.2 ± 0.4	1.3 ± 0.3	1.3 ± 0.3
MUFA total	**48.8 ± 7.4**	**58.8 ± 10.0**	**36.6 ± 7.3**	**62.6 ± 0.1**	**39.5 ± 0.1**	**43.8 ± 10.1**
16:2	0.5 ± 0.2	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
16:3	0.4 ± 0.2	0.6 ± 0.4	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
16:4	0.9 ± 0.6	0.2 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
18:2 (n-6)	1.3 ± 0.2	1.6 ± 0.3	1.2 ± 0.1	1.8 ± 0.1	1.3 ± 0.5	1.4 ± 0.5
18:3 (n-6)	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
18:3 (n-3)	0.7 ± 0.2	0.8 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
18:4 (n-3)	2.2 ± 0.3	1.6 ± 0.6	0.7 ± 0.2	1.3 ± 0.1	0.6 ± 0.4	0.7 ± 0.5
20:2 (n-6)	0.2 ± 0.2	0.2 ± 0.1	0.6 ± 0.9	0.2 ± 0.1	0.3 ± 0.5	0.3 ± 0.5
20:3 (n-6)	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:4 (n-6)	1.0 ± 0.2	0.7 ± 0.4	1.9 ± 0.4	0.7 ± 0.1	1.5 ± 0.4	1.4 ± 0.5
20:3 (n-3)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
20:4 (n-3)	0.8 ± 0.1	0.9 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
20:5 (n-3)	11.9 ± 4.4	6.9 ± 1.2	5.6 ± 0.9	7.3 ± 0.1	6.1 ± 0.9	6.4 ± 0.9
22:4 (n-6)	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.0 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
22:5 (n-6)	0.3 ± 0.0	0.3 ± 0.2	0.9 ± 0.3	0.2 ± 0.1	0.7 ± 0.2	0.7 ± 0.3
22:5 (n-3)	0.7 ± 0.2	0.7 ± 0.2	1.4 ± 0.2	0.6 ± 0.1	1.4 ± 0.4	1.2 ± 0.5
22:6 (n-3)	16.8 ± 2.1	14.7 ± 3.2	20.8 ± 4.4	13.3 ± 0.1	19.3 ± 2.9	18.1 ± 3.6
PUFA total	**38.6 ± 6.9**	**30.2 ± 3.2**	**36.1 ± 6.0**	**28.5 ± 0.2**	**34.6 ± 3.3**	**33.6 ± 4.1**
Fatty alcohols	*B. glaciale* N = 6	*C. microdon* N = 11	*C. pseudopallida* N = 4	*C.*A N = 2	*C.*B N = 12	*Cyclothone* spp. N = 14
14:0	13.9 ± 1.5	13.6 ± 3.8	3.9 ± 2.1	10.2 ± 2.5	4.9 ± 2.3	13.6 ± 5.9.
15:0	1.4 ± 0.3	1.9 ± 0.5	1.7 ± 0.9	1.2 ± 0.3	1.4 ± 0.3	1.8 ± 1.3
16:0	23.3 ± 1.2	20.3 ± 4.3	19.3 ± 5.7	18.3 ± 0.3	22.5 ± 4.1	20.7 ± 21.7
17:0	1.4 ± 0.3	1.3 ± 0.4	1.1 ± 0.3	0.9 ± 0.0	1.1 ± 0.4	1.3 ± 1.1
18:0	7.8 ± 1.5	4.9 ± 1.3	20.0 ± 6.8	4.7 ± 0.1	12.2 ± 5.4	5.2 ± 10.8
20:0	0.6 ± 0.2	0.8 ± 0.3	6.0 ± 1.7	0.6 ± 0.0	3.9 ± 2.1	0.8 ± 3.3
21:0	0.0 ± 0.1	0.0 ± 0.0	3.3 ± 2.9	0.0 ± 0.0	1.8 ± 1.6	0.0 ± 1.5
22:0	0.1 ± 0.0	0.1 ± 0.1	1.3 ± 0.2	0.2 ± 0.1	1.1 ± 0.5	0.1 ± 1.0
16:1(n7)	4.6 ± 0.8	8.6 ± 2.3	0.9 ± 0.2	6.4 ± 0.0	1.9 ± 0.9	8.3 ± 2.7
18:1(n-9)	31.0 ± 4.6	10.0 ± 3.7	8.5 ± 4.3	7.6 ± 1.7	8.7 ± 5.3	9.8 ± 8.5
18:1(n-9)iso	0.0 ± 0.0	2.7 ± 1.4	1.2 ± 0.5	3.3 ± 0.2	1.1 ± 0.7	3.0 ± 1.5
20:1 (n-9)	5.1 ± 1.9	10.9 ± 3.4	5.0 ± 2.5	14.2 ± 0.6	7.4 ± 2.5	10.7 ± 8.6
22:1 (n11)	9.4 ± 3.3	20.7 ± 6.4	19.6 ± 15.0	28.9 ± 0.6	24.5 ± 7.2	20.7 ± 25.3
24:1	1.3 ± 0.5	4.0 ± 1.2	7.9 ± 1.8	3.5 ± 0.4	7.4 ± 2.0	4.0 ± 6.7

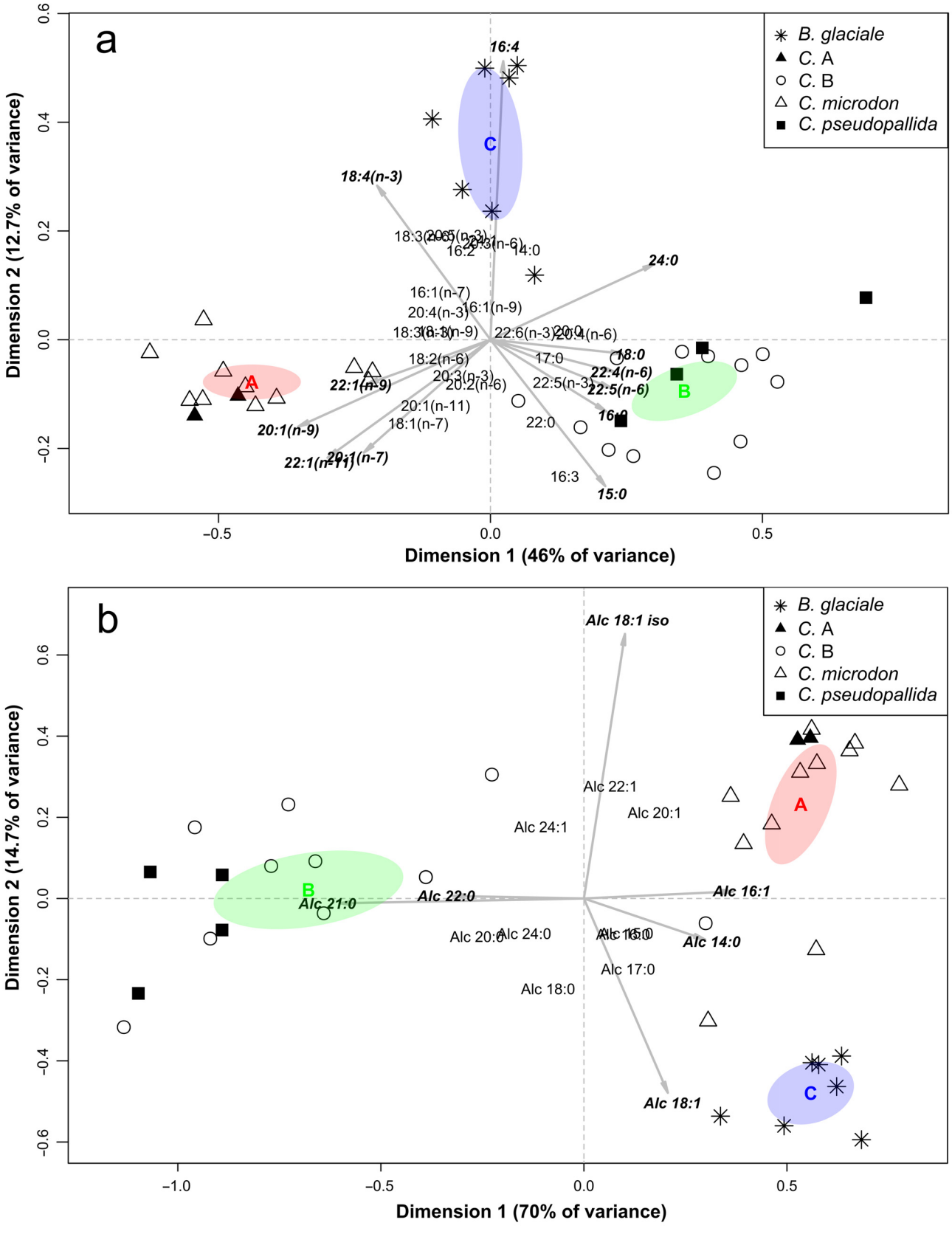

Fig. 2. Mesopelagic and bathypelagic fishes and fatty acids and alcohols plotted against the first 2 dimensions of variance identified by log ratio analysis of (a) fatty acids and (b) fatty alcohols. Major contributors are displayed in ***bold italics***. Centroids and 95% confidence ellipses are shown for 3 groups: Group A (*Cyclothone microdon* + *C.*A), Group B (*C. pseudopallida* + *C.*B) and Group C (*Benthosema glaciale*). Overall group difference was highly statistically significant ($p < 0.00001$) in each analysis

Table 3. Results of 1-way ANOVA of fatty acid trophic markers (FATMs) among 5 groups of meso- and bathypelagic fishes (CM: *Cyclothone microdon*; CA: *Cyclothone* type A; CB: *Cyclothone* type B; CP: *Cyclothone pseudopallida*; BG: *Benthosema glaciale*; df = 4, 31 in all tests) and of Holm-Sidak pairwise comparisons of their means. Shared letters within each FATM indicate no significant difference (at $p < 0.05$) between the groups so marked. Where the data failed variance (18:0, and the ratios of 18:1(n-9)/18:1(n-7), 20:1(n-9)+22:1(n-11), 16:1n7/16:0) or normality tests (fatty acid 16:0), they were log-transformed before ANOVA

FATM	*F*	p	CM	CA	CB	CP	BG
15:0+17:0	58.4	<0.001	a	ac	b	b	c
log 16:0	143.2	<0.001	a	a	b	b	a
log 18:0	86.0	<0.001	a	a	b	b	c
log18:1(n-9)/18:1(n-7)	13.1	<0.001	a	a	a	a	b
22:6(n-3) DHA	9.55	<0.001	a	a	b	b	ab
log 20:1(n-9)+22:1(n-11)	14.3	<0.001	a	a	b	b	b
log 16:1n7/16:0	80.8	<0.001	a	a	b	b	a
16/18 PUFA	11.4	<0.001	a	ab	b	b	ab

sampled at different depths from the surface to 1700 m, there was no evident depth-related variation in FA composition among them.

The FAlc content of *C. microdon*, *C.*A and *B. glaciale* specimens did not include saturated FAlcs (SFAlcs) longer than 20 carbons, while the *C. pseudopallida* specimens had higher proportions of 18 and 20 SFAlcs (Table 2; Fig. S4). The *B. glaciale* specimens contained a high proportion of 18:1(n-9) FAlc, while all *Cyclothone* specimens had high proportions of 20:1(n-9), 22:1(n-11) and 24:1(n-9) alcohols.

3.2. Fatty acid trophic markers

Fig. 3 summarizes the FATMs in each fish specimen that can be attributed to carnivory and bacterial, detrital, copepod and algal sources, the latter indicating feeding on herbivorous zooplankton. Bacterial SFAs (15:0+17:0) were significantly higher (2% of TFAs) in *C. pseudopallida* and *C.*B compared to the other groups (Table 3, Fig. 3a), whereas the latter contained significantly higher proportions (15–20% of TFAs) of each of the FAs attributed to detrital feeding (16:0 and 18:0, Fig. 3a). The composition of the *B. glaciale* specimens showed a mixed carnivory signal. Their 18:1(n-9)/18:1(n-7) FA ratio was elevated (average 16% of TFAs), but their DHA showed the opposite trend. There was no significant difference between the 18:1(n-9)/18:1(n-7) ratio between the remaining groups, but they had significantly lower ratios than those seen in *B. glaciale*. DHA did differ between the *Cyclothone* groups. *C. microdon* and *C.*A had significantly higher proportions (6–10% each) of the 20:1(n-9) and 22:1(n-11) copepod markers (Fig. 3c, Table 3). Those groups and the *B. glaciale* specimens also had significantly higher diatom 16:1(n-7)/16:0 ratios, while *C. pseudopallida* and *C.*B showed elevated dinoflagellate 16/18 PUFA ratios (Fig. 3d).

In analysing our copepod specimens, we focused on the MUFAs 20:1(n-9) and 22:1(n-11), which are usually described as characteristic of the wax-ester-rich copepods of the genus *Calanus*. Of the 18 species analysed, the 3 *Calanus* species indeed had the highest proportions of those 2 MUFAs, at 14–30% of TFAs (Fig. 4). However, the mesopelagic copepod *Lucicutia* had similar levels (17%). The combination of 20:1(n-9) and 22:1(n-11) was about 10% in *Paraeuchaeta* and *Augaptilus* and less than 5% in the remaining copepods.

3.3. Log ratio analysis

The first 2 dimensions identified by LRA of FA composition explained 58.7% of the variance among the specimens (Fig. 2a). The ordination separated them into 3 significantly different groups ($p < 0.00001$), which contained specimens of *B. glaciale*; *C. microdon* and *C.*A; and *C. pseudopallida* and *C.*B, respectively (Fig. 2a). The *B. glaciale* specimens were especially distinct from the other groups in their high content of the phytoplankton trophic markers 16:4 and 18:4(n-3), with minor contributions of 20:5(n-3) and 18:3(n-6). The *C. microdon* group emphasized 20:1(n-9) and 22:1(n-11), markers of lipid-rich calanoid copepods. The bacterial markers 15:0 and 17:0 and the detrital SFA markers 16:0 and 18:0 distinguished the FA profiles of the specimens of *C. pseudopallida.* The PUFAs 20:5(n-6) and 22:5(n-6), also prominent in *C. pseudopallida*, were not used as FATMs.

The first 2 dimensions identified by LRA of FAlc composition captured 84.8% of the variance in the data (Fig. 2b). The differences among the groups, which are consistent with those based on FA content, were statistically significant ($p < 0.0001$). The *B. glaciale* group was separated from the others by the 18:1 FAlc, while SFAlc 21:0 shaped the distinction of the *C. pseudopallida* + *C.*B combination. No one FAlc characterized the *C. microdon* + *C.*A group. Rather, this group was distinguished by a combination of 20:1, 16:1 and 18:1-iso.

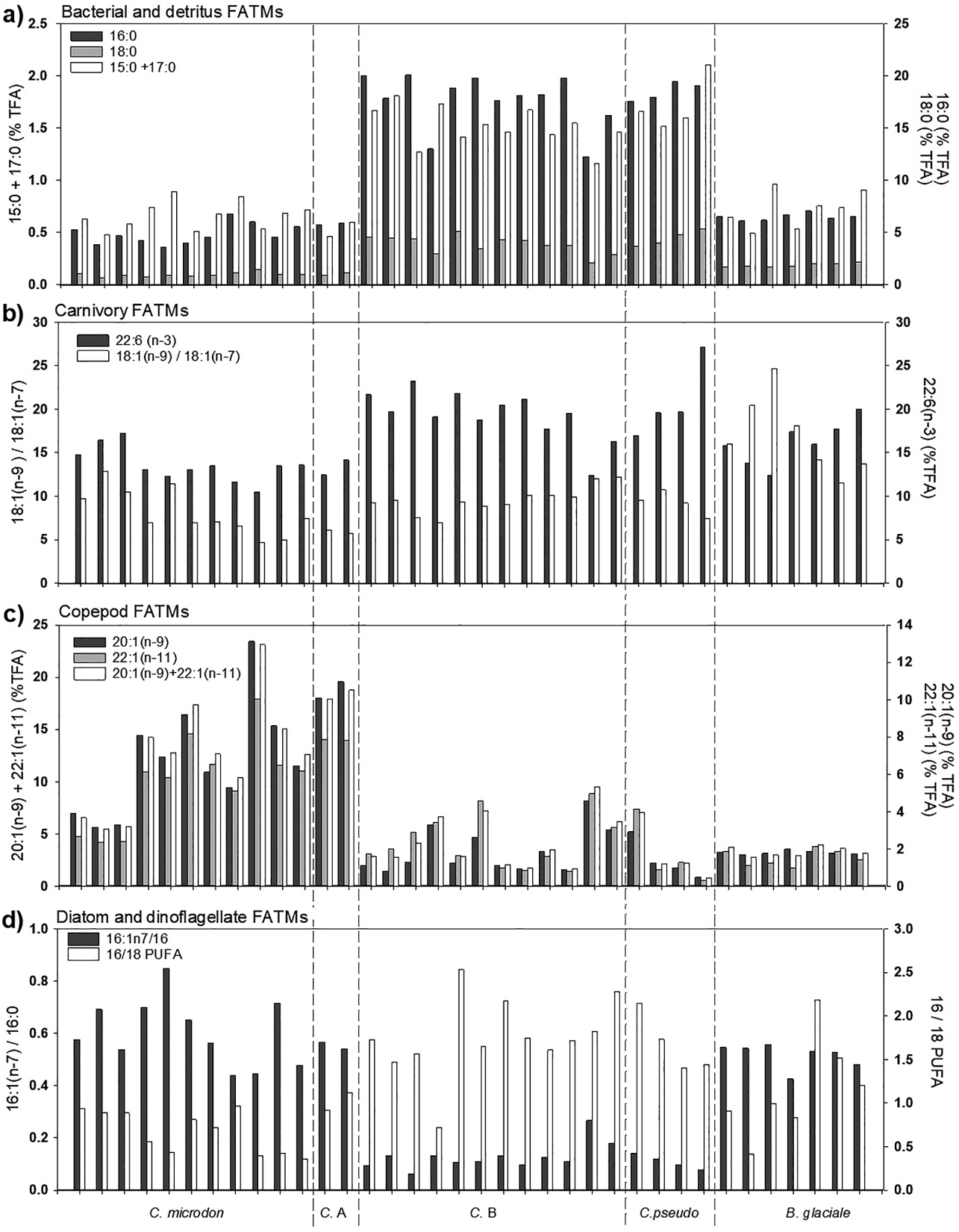

Fig. 3. Fatty acid trophic markers (FATMs) of the 37 meso- and bathypelagic fish, each plotted as % of total FA (TFA) content or ratios. (a) Bacterial biomarkers: sum of the odd-chained FAs 15:0 and 17:0 (Perry et al. 1979), and detrital biomarkers 16:0 and 18:0 FAs (Zhukova 2019). (b) Carnivorous biomarkers 18:1(n-9)/18:1(n-7) (Sargent & Falk-Petersen 1981, Falk-Petersen et al. 2000) and 22:6(n-3) (Stevens et al. 2004). (c) Calanid copepod markers 20:1(n-9) and 22:1(n-11) and their sum (Sargent & Falk-Petersen 1981, Hagen et al. 1995). (d) Diatom and dinoflagellate biomarkers 16:1n7/16:0 (Cripps et al.1999) and 16:18 polyunsaturated FAs (Dalsgaard et al. 2003). *C.*A and *C.*B are the unknown *Cyclothone* individuals that grouped with *C. microdon* and *C. pseudopallida*, respectively, in the log ratio analysis. Statistics of the differences between the groups are presented in Table 3

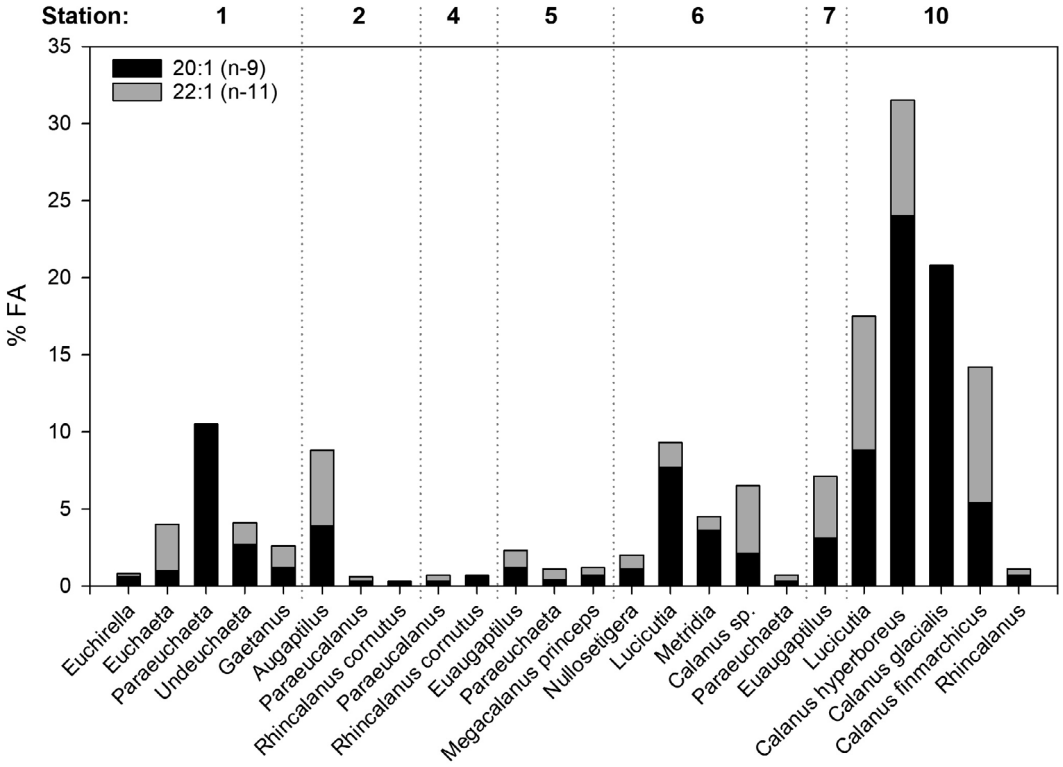

Fig. 4. Proportions of the long-chain monounsaturated fatty acids 20:1(n-9) and 22:1(n-11) in 23 samples of copepods, presented by taxon and station. Copepods were not analyzed from Stn 3

4. DISCUSSION

We found trophic markers indicative of specific components of the recent (weeks to months) dietary history of our meso- and bathypelagic fish specimens. Those markers suggest 3 different pathways of vertical transport of organic carbon from the epipelagic zone. First was a direct link to the surface shown by the phytoplankton markers, which indicate predation on surface-feeding herbivorous zooplankton. Second, there were markers indicating feeding on deeper-living copepods (e.g. *Lucicutia*) and third, FAs indicating either a dietary input of marine snow or secondary consumption of zooplankton that fed on marine snow. While these findings are indicative of the dietary input of the fish we analysed, more sampling across larger spatio-temporal scales would be needed to resolve variations across habitats and local prey availabilities before drawing general conclusions about the diets of the 3 fish species analysed here.

Despite the high abundance and ecological importance of meso- and bathypelagic fishes, their trophic ecology remains poorly understood. Fewer than 25 studies, globally, have reported on the FA composition of any of them, and of these, only 6 have addressed North Atlantic species. These 6 studies are from 4 main areas: the northern Norwegian Sea and around Svalbard (Falk-Petersen et al. 1986, Geoffroy et al. 2019, Olsen et al. 2020), the Iceland Basin (Petursdottir et al. 2008), the northwest Atlantic off Newfoundland, Canada (Parzanini et al. 2018), and off the Canary Islands (Culkin & Morris 1970). Of the species examined here, only the Norwegian Sea and Iceland Basin studies reported on *Benthosema glaciale*, and only the work off Newfoundland considered *Cyclothone microdon*. None of these studies, including ours, addressed seasonality in diets.

Our study covered a depth-resolved transect from the tropical to the temperate North Atlantic (Fig. 1). Specimens of *Cyclothone* were collected at all stations. All specimens confirmed as *C. pseudopallida* were from 1 station at the southern part of the sampling transect and were captured either at 800–1200 m depth in daylight or at 200–500 m at night. Estimates of the biomass of *C. pseudopallida* from the same cruise indicate that the species is mesopelagic, inhabiting waters above 1000 m depth (Sarmiento-Lezcano et al. 2022). Our *C.*B specimens were also from the southern part of the transect, taken at Stns 1–4, captured either in the upper 700 m at night or at bathypelagic depths during daylight, with 1 specimen taken below 1600 m depth. Their similarity in FA content grouped them with the *C. pseudopallida* specimens, having similar feeding modes that emphasized marine snow and a bacteria-based

diet. However, the biomass distribution reported by Sarmiento-Lezcano et al. (2022) suggests that the *C.*B were probably not all *C. pseudopallida*. In contrast, the confirmed *C. microdon* were captured at the northern stations, generally between 800 and 1700 m depth with 1 near-surface specimen. This *C. microdon* specimen at the surface is likely to have entered the surface net at depth, as Sarmiento-Lezcano et al. (2022) reported *C. microdon* to be present only at bathypelagic depths. The FA composition of *C. microdon* suggests feeding on lipid-rich mesopelagic zooplankton. In their spatial distribution, the *C.*A specimens overlapped with *C.*B at Stn 4 but were taken from different depth layers (Fig. S1). Individuals captured at night in the mesopelagic (400–700 m) layer bore the markers of a zooplankton diet, whereas those taken in the bathypelagic zone during daylight had a composition suggesting a detritivorous feeding mode. Five *Cyclothone* species were identified in the expedition catches (Sarmiento-Lezcano et al. 2022), and our unidentified *C.*A and *C.*B, which were defined by feeding modes rather than taxonomy, could include members of any of those.

The different trophic pathways of the 2 *Cyclothone* groups, reflected in their FA compositions, could have arisen from different feeding modes employed by the various species or else from differing dietary environments along the survey transect. The second explanation seems more likely, as opportunistic feeding is common in resource-deficient meso- and bathypelagic ecosystems (Bernal et al. 2015). Thus, it is notable that the geographical location of the 2 trophic groups is separated by the Mediterranean outflow, which may divide the ecosystems south of Stn 5 from those north of Stn 4. Our study indicates that the northern ecosystem supports *Cyclothone* primarily by a dietary path from phytoplankton through copepods/zooplankton, whereas the southern one supplies those species more by the flux of sinking carbon.

4.1. FATM-based groups

Meso- and bathypelagic fishes, particularly those from the family Myctophidae, have been characterized as opportunistic feeders, preying on a variety of zooplankton, including ichthyoplankton, while some are partially or fully piscivorous (Bernal et al. 2015).

4.1.1. *Benthosema glaciale*

The relatively large intra-specific variance observed in the FATMs of our *B. glaciale* specimens (Fig. 3) can be explained by a broad food spectrum (García-Seoane et al. 2013, (Papadimitraki et al. 2023). The specimens were all captured at the same station, near the surface at night. High levels of diatom markers and markers of carnivory such as 18:1(n-9)/18:1(n-7) support the notion of an opportunistic feeding strategy. Large amounts of 20:1(n-9) and 22:1(n-11) FAs (15–19% of TFAs) have been observed in *B. glaciale* from both the Norwegian Sea and Iceland Basin (Falk-Petersen et al. 1986, Petursdottir et al. 2008, Geoffroy et al. 2019, Olsen et al. 2020). These values are higher than the 4% observed in our study, indicating that those specimens had a different diet, likely based on *Calanus* which dominates the copepod biomass in the subarctic Norwegian and Iceland Basin waters (Gislason 2008, Broms et al. 2009). The lipid content, 3% of wet weight, that we observed was lower than the 6% that Petursdottir et al. (2008) found in the Iceland Basin. Our *B. glaciale* specimens had the most pronounced 18:1 FAlc composition, coinciding with the phytoplankton FATM in the LRA plot.

4.1.2. *Cyclothone microdon*

The FA compositions of the specimens of *C. microdon* in our study included relatively high but variable (6–23% of TFAs) amounts of the calanoid markers 20:1(n-9) and 22:1(n-11), indicating a feeding strategy focused on wax-ester-rich copepods. Considering the relatively deep capture depth of the *C. microdon* specimens, we presume these markers could result from predation on the co-occurring mesopelagic copepods of the genus *Lucicutia* that were characterized by high proportions of these FATMs. *Lucicutia* was sampled at 700–1000 m depth at Stn 6 and at 1300–1600 m depth at Stn 10, coinciding with specimens of *C. microdon* at both stations.

The only comparable study of *C. microdon* was based on 2 specimens collected in winter off Newfoundland (Parzanini et al. 2018). The FATM profile of those specimens was very similar to ours, though with much higher levels of calanoid markers (37%). In that region, *Calanus finmarchicus* and *C. hyperboreus* dominate the copepod community (Head & Sameoto 2007). The phytoplankton markers in the Newfoundland specimens were at similar levels to those seen in the *C. microdon* and *C.*A specimens of the present study, but with reverse signals of dinoflagellate rather than diatom FATMs.

4.1.3. *Cyclothone pseudopallida*

Our specimens of *C. pseudopallida* contained markers indicative of a trophic pathway involving detritus, with detrital biomarkers comprising over 20% of TFAs, as well as carnivory, based on high DHA (not 18:1(n-9)), and an elevated dinoflagellate signal. Along with the bacterial markers, this composition suggests a dietary input of marine snow or predation on organisms that consume marine snow. Odd-chain FAs, such as 15:0 and 17:0, generally constitute under 1.5% of the TFAs of meso- and bathypelagic fishes (Culkin & Morris 1970, Kayama & Ikeda 1975, Nevenzel & Menon 1980) while falling between 1.5 and 2% in the *C. pseudopallida* and *C.*B specimens in the present study. The link between these markers and the trophic ecology of meso- and bathypelagic fishes has not previously been reported. Nonetheless, the *C. pseudopallida* and *C.*B specimens we analysed contained more than double the amount of 15:0 of the closely related *C. microdon* specimens and more than 3 times the amount we found in specimens of *B. glaciale*, which was also the case for the 16:0 FA content.

It is noteworthy that the 2 trophic markers assigned to carnivory, the 18:1(n-9)/18:1(n-7) ratio and DHA, do not always coincide. In our *C. microdon* and *C.*A specimens, the markers were similar, while in the other groups they were contrasting (Fig. 3b). All sampled fish are likely carnivorous at some trophic level, preying on zooplankton from herbivores to carnivorous copepods, amphipods and others. However, the pattern shown here is worth further investigation, as the 2 markers indicate predation at different trophic levels.

It should also be noted that the analyses were conducted on whole fish, not specific targeted tissues. FAs in organisms are found in membranes, tissues and storage lipids, and if retention of a specific dietary FA differs between lipid-rich and lipid-poor organisms, it could bias the use of FATMs and species comparisons. However, in the present study, all fish had low lipid content (Table 1) and some wax ester storage, and dietary lipids were represented in both tissues and lipid stores. FATMs also specifically take into account membrane lipids, as 22:6 (n-3) is retained and is therefore used as an FATM for carnivory, and 18:1(n-9) is similarly produced by organisms and is also used in the ratio with phytoplankton lipid (18:1(n7)) as a marker of carnivory.

4.2. Fatty alcohol composition

The presence of FAlc indicates storage of wax esters. Wax esters are used by mesopelagic fishes for both energy storage and buoyancy (Nevenzel 1970). *B. glaciale* is reported to have about 56–80% of its lipids as wax esters (Falk-Petersen et al. 1986, Geoffroy et al. 2019, Olsen et al. 2020), while *C. pseudopallida* has 17–53% of its total lipids as wax esters (Kayama & Ikeda 1975, Nevenzel & Menon 1980). No estimates of lipid type are available for *C. microdon*, but the presence of FAlc in our specimens indicates that some fraction of *C. microdon* lipids are indeed wax esters.

FAlcs are presumed to be synthesized de novo by organisms from dietary amino acids and glucose (Sargent et al. 1981) and, therefore, they are not lipid-based dietary markers. It is, however, clear that the fish in our study follow different paths in FAlc production, with *B. glaciale* specimens making 16:0 and 18:1 FAlc, while specimens of *C. microdon* and *C.*A generate 16:0 and 22:1, and *C. pseudopallida* and *C.*B specimens generate the 18:0 and 22:1 combination. The calanoid tracer FAlcs and FAs, 20:1 and 22:1, fall within the same fish groups in the LRA analyses (Fig. 2). From the limited available data, it is unclear whether the FAlc composition reflects different life history traits of *B. glaciale* and the *Cyclothone* species, but our results provide reason for further investigation of wax ester structure and life history of meso- and bathypelagic fishes.

4.3. *Cyclothone* feeding ecology

As *Cyclothone* spp. are weak-bodied, lack the bioluminescent lures seen in some meso- and bathypelagic fishes and do not undertake DVM, they appear adapted to relying on chance encounters in an environment of low prey availability. That could drive a foraging strategy which minimizes energy use and maximizes the utilization of ingested prey. A high proportion of empty stomachs and almost none with more than a single prey item have been reported by a number of studies of members of the genus (e.g. Collard 1970, Dewitt & Cailliet 1972, Roe & Badcock 1984, Thompson & Kenchington 2017), indicating that *Cyclothone* endure periods of starvation between meals. *Cyclothone* also exhibit prey selectivity in some cases, but the variable diets may reflect variances in local prey availability rather than selective foraging (Thompson & Kenchington 2017). The mesopelagic copepod *Pleuromamma* spp. is one of the principal constituents of *Cyclothone* diets in open-ocean surveys (e.g. Roe & Badcock 1984, Hopkins et al. 1996, Yoon et al. 2007). Despite that, continental shelf zooplankton species dominated the diet

of *C. microdon* collected in a submarine canyon incised into a continental shelf, whereas open-ocean species were only observed in few specimens there (Thompson & Kenchington 2017). The proportional contribution of the calanoid copepod markers 20:1(n-9) and 22:1(n-11) to the FA profile of specimens of *C. microdon* in the present study, and the high content of these markers in coinciding species such as deep-living *Lucicutia* sp., supports the hypothesis that lipid-rich copepods represent important constituents of the diet of *C. microdon*.

The advantage of conserving energy by avoiding the effort of DVM is likely countered by the disadvantage of relying on diel migrants, which become less abundant as depth increases. This may drive a more omnivorous feeding strategy which includes macroscopic particulate organic matter (POM), in the form of marine snow particles. This effect could explain the observed differences between our specimens of *C. pseudopallida*, in which the relatively higher proportions of odd-number SFAs, characteristic of dead organic matter, drive the differentiation from the other 2 groups. Gut content studies place *C. pseudopallida* in a copepod-feeding guild along with closely related *C. alba* and *C. braueri* (McClain-Counts 2017). However, marine snow is not easily identified in gut contents, and most of the specimens reported in the literature had empty stomachs. A possible explanation could be that some of the diet of *C. pseudopallida* may originate from detritus and that the same could be the case for *C. alba* and *C. braueri*, although this is speculative due to the current limited knowledge about these species. The relatively high proportion of odd-number SFAs does not necessarily mean that the specimens in the *C. pseudopallida* group feed directly on POM or detritus, as they may in fact be feeding on zooplankton or gelatinous plankton which in turn have been feeding on detrital matter. The reported peak abundances at depths of 300–900 m for *C. pseudopallida* (Shinohara et al. 1994, Sarmiento-Lezcano et al. 2022) and 500–2700 m (Mauchline 1988) should enable *C. pseudopallida* to prey on mesopelagic copepods, rather than rely on marine snow or detritus, and counterintuitively, detritus seems to play a lesser role in the dietary input of the generally deeper-dwelling *C. microdon*. The organic compounds that are bio-synthesized de novo by bacteria during the decomposition of organic matter in marine snow can be accessed directly by meso- and bathypelagic fishes, which would make them at least in part detritivorous, but they may also be accessed indirectly by consuming filter-feeding organisms (Choy et al. 2015), which would make them carnivorous.

In summary, our findings are largely in agreement with the current literature on the diet of *C. microdon*, *C. pseudopallida* and *B. glaciale*. However, the results also indicate that detritus or detritivorous suspension feeding constituted a significant part of the diet of the *C. pseudopallida* specimens we analysed, which has not been reported for this species before.

5. CONCLUSION

The application of integrative indicators, including FATMs, to understand the diets of cryptic and inaccessible fishes in the meso- and bathypelagic zone can provide insight into trophic links between groups of organisms in the deep sea. We found that the lipid profiles and relative proportions of characteristic trophic markers clearly differed between specimens of 3 north Atlantic species of meso- and bathypelagic fish, including the first complete lipid profile of 4 specimens of *Cyclothone pseudopallida.* Individuals belonging to the species *Benthosema glaciale, C. microdon* and *C. pseudopallida* each contained characteristic markers of diatoms, calanoid copepods and bacteria, respectively, indicating clear differences in dietary input.

Data availability. The data used in the article is available at Jónasdóttir SH, Maar K, Fonseca CT, Pérez-Jorge S, Silva MA (2022): Compilation of fatty acid composition of marine biota from the central and Northeast Atlantic, and the Mediterranean Sea. PANGAEA, https://doi.org/10.1594/PANGAEA.945881

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LITERATURE CITED

Aitchison J (1990) Relative variation diagrams for describing patterns of compositional variability. Math Geol 22: 487–511

Bar-On YM, Phillips R, Milo R (2018) The biomass distribution on Earth. Proc Natl Acad Sci USA 115:6506–6511

Bernal A, Olivar MP, Maynou F, Fernández de Puelles ML (2015) Diet and feeding strategies of mesopelagic fishes in the western Mediterranean. Prog Oceanogr 135:1–17

Broms C, Melle W, Kaartvedt S (2009) Oceanic distribution and life cycle of *Calanus* species in the Norwegian sea and adjacent waters. Deep Sea Res II 56:1910–1921

Choy AC, Popp BN, Hannides CCS, Drazen JC (2015) Trophic structure and food resources of epipelagic and mesopelagic fishes in the north Pacific subtropical gyre ecosystem inferred from nitrogen isotopic compositions. Limnol Oceanogr 60:1156–1171

Collard SB (1970) Forage of some eastern Pacific midwater fishes. Copeia 1970:348–354

Cripps GC, Watkins JL, Hill HJ, Atkinson A (1999) Fatty acid content of Antarctic krill *Euphausia superba* at South Georgia related to regional population and variations in diet. Mar Ecol Prog Ser 181:177–188

Culkin F, Morris RJ (1970) The fatty acids of some marine teleosts. J Fish Biol 2:107–112

Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Davison PC, Checkley DM Jr, Koslow JA, Barlow J (2013) Carbon export mediated by mesopelagic fishes in the northeast Pacific Ocean. Prog Oceanogr 116:14–30

Dewitt FA Jr, Cailliet GM (1972) Feeding habits of two bristlemouth fishes, *Cyclothone acclinidens* and *C. signata* (Gonostomatidae). Copeia 1972:868–871

Falk-Petersen I, Falk-Petersen S, Sargent JR (1986) Nature, origin and possible roles of lipid deposits in *Maurolicus muelleri* (Gmelin) and *Benthosema glaciale* (Reinhart) from Ullsfjorden, northern Norway. Polar Biol 5:235–240

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57(s3):178–191

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509

Galloway AWE, Budge SM (2020) The critical importance of experimentation in biomarker-based trophic ecology. Philos Trans R Soc B 375:20190638

García-Seoane E, Dalpadado P, Vázquez A (2013) Feeding ecology of the glacier lanternfish *Benthosema glaciale* (Actinopterygii, Myctophidae) in the Flemish Cap (north Atlantic Ocean). Hydrobiologia 717:133–146

Gartner JV, Hopkins TL, Baird RC, Miliken DM (1987) The lanternfishes (Pisces, Myctophidae) of the eastern Gulf of Mexico. Fish Bull 85:81–98

Geoffroy M, Daase M, Cusa M, Darnis G and others (2019) Mesopelagic sound scattering layers of the high Arctic: seasonal variations in biomass, species assemblage, and trophic relationships. Front Mar Sci 6:364

Gibbs RH Jr, Krueger WH (eds) (1987) Biology of midwater fishes of the Bermuda Ocean Acre. Smithson Contrib Zool 452:1–187

Gislason A (2008) Vertical distribution and seasonal dynamics of mesozooplankton in the Iceland Basin. Mar Biol Res 4:401–413

Gjøsaeter J, Kawaguchi K (1980) A review of the world resources of mesopelagic fish. Fish Tech Pap 193. FAO, Rome

Graeve M, Greenacre MJ (2020) The selection and analysis of fatty acid ratios: a new approach for the univariate and multivariate analysis of fatty acid trophic markers in marine pelagic organisms. Limnol Oceanogr 18:196–210

Graeve M, Hagen W, Kattner G (1994) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep Sea Res I 41:915–924

Greenacre M (2016) Data reporting and visualization in ecology. Polar Biol 39:2189–2205

Greenacre M (2018) Compositional data analysis in practice. Chapman and Hall/CRC Press, Boca Raton, FL

Greenacre M (2021) Compositional data analysis. Annu Rev Stat Appl 8:271–299

Greenacre M, Groenen PJF, Hastie T, D'Enza AI, Markos A, Tuzhilina E (2022) Principal component analysis. Nat Rev Methods Primers 2:100

Hagen W, Kattner G, Graeve M (1995) On the lipid biochemistry of polar copepods: compositional differences in the Antarctic calanoids *Euchaeta antarctica* and *Euchirella rostromagna*. Mar Biol 123:451–457

Head EJH, Sameoto DD (2007) Inter-decadal variability in zooplankton and phytoplankton abundance on the Newfoundland and Scotian shelves. Deep Sea Res II 54: 2686–2701

Hopkins TL, Sutton TT, Lancraft TM (1996) The trophic structure and predation impact of a low latitude midwater fish assemblage. Prog Oceanogr 38:205–239

Hulley PA (1981) Results of the research cruise of FRV Walter Herwig to South America. Family Myctophidae (Osteichthyes, Myctophiformes). Arch FischWiss 32:1–300

Irigoien X, Klevjer TA, Røstad A, Martinez U and others (2014) Large mesopelagic fishes biomass and trophic efficiency in the open ocean. Nat Commun 5:3271

Jónasdóttir SH (2019) Fatty acid profiles and production in marine phytoplankton. Mar Drugs 17:151

Kattner G, Hagen W (1995) Polar herbivorous copepods — different pathways in lipid biosynthesis. ICES J Mar Sci 52:329–335

Kayama M, Ikeda Y (1975) Studies on the lipids of micronectonic fishes caught in Sagami and Suruga Bays, with special reference to their wax esters. J Jpn Oil Chem Soc 24: 435–440

Mauchline J (1988) Growth and breeding of meso- and bathypelagic organisms of the Rockall Trough, northeastern Atlantic Ocean and evidence of seasonality. Mar Biol 98:387–393

McClain-Counts JP, Demopoulos AWJ, Ross SW (2017) Trophic structure of mesopelagic fishes in the Gulf of Mexico revealed by gut content and stable isotope analyses. Mar Ecol 38:e12449

Mehner T, Kasprzak P (2011) Partial diel vertical migrations in pelagic fish. J Anim Ecol 80:761–770

Meillat M (2012) Essais du chalut mésopélagos pour le programme MYCTO 3D – MAP de l'IRD, à bord du Marion Dufresne. IFREMER Rapport de mission Marion Dufresne. R.INT.RBE/STH/LTH 2012-05

Merrett NR, Roe HSJ (1974) Patterns and selectivity in the feeding of certain mesopelagic fishes. Mar Biol 28: 115–126

Nelson JS (2006) Fishes of the world, 4th edn. John Wiley & Sons, Hoboken, NJ

Nevenzel JC (1970) Occurrence, function and biosynthesis of wax esters in marine organisms. Lipids 5:308–319

Nevenzel JC, Menon NK (1980) Lipids of midwater marine fish: family Gonostomatidae. Comp Biochem Physiol B Comp Biochem 65:351–355

Oksanen J, Blanchet BF, Friendly M, Kindt R and others (2020) vegan: community ecology package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Olivar PM, Bode A, López-Pérez C, Hulley AP, Hernández-León S (2019) Trophic position of lanternfishes (Pisces: Myctophidae) of the tropical and equatorial Atlantic estimated using stable isotopes. ICES J Mar Sci 76:649–661

Olsen RE, Strand E, Melle W, Nørstebø JT and others (2020) Can mesopelagic mixed layers be used as feed sources for salmon aquaculture? Deep Sea Res II 180: 104722

Papadimitraki M, Maar K, Jonasdottir SH (2023) Meso- and bathypelagic fish feeding ecology: a meta-analysis on fatty acids and stable isotope trophic studies. Deep Sea Res I 198:104083

Parzanini C, Parrish CC, Hamel JF, Mercier A (2018) Functional diversity and nutritional content in a deep-sea faunal assemblage through total lipid, lipid class, and fatty acid analyses. PLOS ONE 13:e0207395

Peña M, Cabrera-Gámez J, Domínguez-Brito AC (2020) Multi-frequency and light-avoiding characteristics of deep acoustic layers. Mar Environ Res 154:104842

Perry GJ, Volkman JK, Johns RB, Bavor HJ Jr (1979) Fatty acids of bacterial origin in contemporary marine sediments. Geochim Cosmochim Acta 43:1715–1725

Petursdottir H, Gislason A, Falk-Petersen S, Hop H, Svavarsson J (2008) Trophic interactions of the pelagic ecosystem over the Reykjanes Ridge as evaluated by fatty acid and stable isotope analyses. Deep Sea Res II 55: 83–93

Pinti J, DeVries T, Norin T, Serra-Pompei C and others (2023) Model estimates of metazoans' contributions to the biological carbon pump. Biogeosciences 20:997–1009

Potier M, Marsac F, Cherel Y, Lucas V, Sabatié R, Maury O, Ménard F (2007) Forage fauna in the diet of three large pelagic fishes (lancetfish, swordfish and yellowfin tuna) in the western equatorial Indian ocean. Fish Res 83: 60–72

Roe HSJ, Badcock J (1984) The diel migrations and distributions within a mesopelagic community in the northeast Atlantic. 5. Vertical migrations and feeding of fish. Prog Oceanogr 13:389–424

Saint-Béat B, Baird D, Asmus H, Asmus R and others (2015) Trophic networks: How do theories link ecosystem structure and functioning to stability properties? A review. Ecol Indic 52:458–471

Sargent JR, Falk-Petersen S (1981) Ecological investigations on the zooplankton community in Balsfjorden, northern Norway: lipids and fatty acids in *Meganyctiphanes norvegica, Thysanoessa raschi* and *T. inermis* during midwinter. Mar Biol 62:131–137

Sargent JR, Gatten RR, Henderson R (1981) Marine wax esters. Pure Appl Chem 53:867–871

Sarmiento-Lezcano AN, Olivar MP, Peña M, Landeira JM and others (2022) Carbon remineralization by small mesopelagic and bathypelagic Stomiiforms in the Northeast Atlantic Ocean. Prog Oceanogr 203:102787

Shinohara G, Yabe K, Nakaya M, Anma G, Yamaguchi S, Amaoka K (1994) Deep-sea fishes collected from the north Pacific by the T/S Oshoro-Maru. Bull Fac Fish Hokkaido Univ 45:48–80

Silva MA, Fonseca CT, Olivar MP, Bernal A and others (2022) MesopTroph, a database of trophic parameters to study interactions in mesopelagic food webs. Sci Data 9: 716

Spitz J, Mourocq E, Leauté JP, Quéro JC, Ridoux V (2010) Prey selection by the common dolphin: fulfilling high energy requirements with high quality food. J Exp Mar Biol Ecol 390:73–77

St. John MA, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar Ecol Prog Ser 131: 75–85

St. John MA, Borja A, Guillem C, Heath M and others (2016) A dark hole in our understanding of marine ecosystems and their services: perspectives from the mesopelagic community. Front Mar Sci 3:31

Stevens CJ, Deibel D, Parrish CC (2004) Copepod omnivory in the North Water Polynya (Baffin Bay) during autumn: spatial patterns in lipid composition. Deep Sea Res I 51: 1637–1658

Suntsov AV, Brodeur RD (2008) Trophic ecology of three dominant myctophid species in the northern California current region. Mar Ecol Prog Ser 373:81–96

Sutton TT (2013) Vertical ecology of the pelagic ocean: classical patterns and new perspectives. J Fish Biol 83: 1508–1527

Thompson SE, Kenchington TJ (2017) Distribution and diet of *Cyclothone microdon* (Gonostomatidae) in a submarine canyon. J Mar Biol Assoc UK 97:1573–1580

Trueman CN, Johnston G, O'Hea B, MacKenzie KM (2014) Trophic interactions of fish communities at midwater depths enhance long-term carbon storage and benthic production on continental slopes. Proc R Soc B 281: 20140669

Watanabe H, Masatoshi M, Kaqaguchi K, Ishimaru K, Ohno A (1999) Diel vertical migration of myctophid fishes (family Myctophidae) in the transitional waters of the western north Pacific. Fish Oceanogr 8:115–127

Watanuki Y, Thiebot JP (2018) Factors affecting the importance of myctophids in the diet of the world's seabirds. Mar Biol 165:79

Yoon WD, Nival P, Choe SM, Picheral M, Gorsk YG (2007) Vertical distribution and nutritional behavior of *Cyclothone braueri, Nematoscelis megalops, Meganyctiphanes norvegica* and *Salpa fusiformis* in the NW Mediterranean mesopelagic zone. ICES CM 2007/F:03. ICES, Copenhagen

Zhukova NV (2019) Fatty acids of marine mollusks: impact of diet, bacterial symbiosis and biosynthetic potential. Biomolecules 9:857

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