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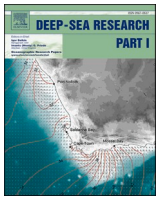

In situ diet patterns and health status of cold-water corals in the Lacaze-Duthiers canyon (NW Mediterranean Sea): insights from fatty acid biomarkers on lipid classes

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ABSTRACT

Hydrodynamic and food supply favourable conditions in the Lacaze-Duthiers Canyon (northwestern Mediterranean Sea) have supported the establishment of deep-sea corals, with some of the highest densities recorded in the Mediterranean. However, increasing pressures on these vulnerable habitats raise critical questions about their ecological status and resilience to environmental changes, emphasising the need to understand their trophic ecology. This study provides the most detailed analysis to date of the lipid profiles and fatty acid signatures of two key framework-building cold-water corals, *Madrepora oculata* and *Desmophyllum pertusum* (syn. *Lophelia pertusa*), offering insights into their feeding strategies, dietary requirements, and nutritional condition.

The results reveal that lipid classes exhibit distinct fatty acid compositions based on functional roles (storage vs. structural). Fatty acid trophic markers (FATM) indicative of herbivorous calanoids, carnivorous copepods, and phytodetritus were detected in the storage lipids (waxes and triglycerides) of both species, yet no FATM was specific to either species. This indicates a mixed diet and dietary overlap between the two corals. However, the composition of storage lipids varied significantly across samples, likely reflecting (1) species-specific feeding preferences, with *D. pertusum* showing greater reliance on overwintering copepods, and (2) the dynamic availability of food resources within the canyon.

Phospholipids were enriched in polyunsaturated fatty acids (PUFA), suggesting that dietary inputs are of sufficiently high quality to meet the metabolic demands of cold-water corals. High levels of storage lipids, primarily long-term reserves (wax esters) enriched in PUFA and zooplankton markers, underscore the good nutritional status of cold-water corals in the Lacaze-Duthiers Canyon.

1. Introduction

The perception of cold-water corals has evolved significantly since the early 20th century, when they were considered as "harmful for trawlers" (Joubin, 1922). Today, the ecological importance of these foundation species and the ecosystem services they provide are widely recognised and undisputed. Cold-water corals are deep-water, framework-building scleractinians inhabiting a wide depth range down to >3000 m (Roberts et al., 2009). Thriving in the absence of light, these corals do not form symbiotic associations with zooxanthellae, relying instead on the direct supply of food (Freiwald et al., 2004). Cold-water corals feed passively on suspended particles, using stinging tentacles (Mortensen, 2001) or mucus nets to capture a wide range of prey drifting in the deep currents (Murray et al., 2019; Zetsche et al., 2016). Consequently, they are often found in areas with high hydrodynamic conditions and increased surface productivity (Roberts et al., 2009). Like their tropical counterparts, these bio-engineers create complex three-dimensional habitats, forming unique biodiversity hotspots (Freiwald et al., 2004; Roberts et al., 2009).

Extensive exploration over the past two decades has revealed that cold-water coral reefs are widespread in all oceans, including the Mediterranean Sea where new reef areas have been discovered recently (Gori et al., 2023) underscoring their broad, but more fragmented, distribution in this region (Chimienti et al., 2019). This patchiness is driven by a

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combination of suitable abiotic and biotic conditions, including topography, water mass properties, and trophic carrying capacity (Gori et al., 2023). Well-developed cold-water coral communities have been discovered in diverse environments such as escarpments, submarine canyons, seamounts, and outer continental shelves and slopes (Gori et al., 2023). In the Gulf of Lion (French continental margin of the NW Mediterranean Sea), communities dominated by the white corals *Madrepora oculata* and *Desmophyllum pertusum* (syn. *Lophelia pertusa*) are found on the flanks of submarine canyons that incise the continental shelf down to more than 2000m (Chimienti et al., 2019; Fabri et al., 2014; Gori et al., 2013; Puig and Gili, 2019). These ecosystem engineers create complex biogenic structures that host rich faunal assemblages, providing feeding, sheltering, reproductive, and nursery grounds for numerous species, including those of commercial importance (Capezzuto et al., 2018; D'Onghia et al., 2017; Rueda et al., 2019).

Cold-water coral habitats are however highly vulnerable to anthropogenic threats such as destructive fishing practices (bottom trawling and longlining), increasing pollution, and ongoing climate change (D'Onghia et al., 2017; Freiwald et al., 2004; Maier et al., 2019). Among the many factors that determine the ability of an organism to acclimate to new environmental conditions and cope with stress, its nutritional status plays a critical role, as tolerance has an energetic cost (Biagianti-Risbourg et al., 2013). Experimental studies have shown that adequate feeding increases the resilience of tropical corals to acidification and warming (Edmunds, 2011; Towle et al., 2015). There are also indications that high food availability may similarly help cold-water corals compensate for these effects (Büscher et al., 2017; Martínez-Dios et al., 2020). Given that global warming is expected to impair the export of organic particles to the deep Mediterranean basins, a precise assessment of the current trophic status of Mediterranean cold-water corals is crucial. Trophic status, defined here as the level and composition of tissue reserves, may serve as a proxy for physiological resilience. These reserves result from the integration of recent dietary inputs, both in terms of quantity and quality, and reflect the coral's ability to meet its nutritional and metabolic requirements (e.g., Maier et al., 2019). Not only does the amount of stored energy determine the capacity to sustain essential functions such as maintenance, growth, and calcification during periods of food scarcity or stress, but the nature of these reserves also affects how efficiently they can be mobilised and utilised. A sustained decline in food inputs may thus lead to depleted reserves, impaired physiological function, and reduced resilience, ultimately compromising the long-term persistence of coral populations under future environmental change.

Experimental studies have shown that cold-water corals are opportunistic suspension-feeders that can exploit a large array of food types, including zooplankton, phytoplankton, phytodetritus, bacteria and dissolved organic matter (Da Ros et al., 2022; Gori et al., 2014; Mueller et al., 2014; Van Oevelen et al., 2016). *Desmophyllum pertusum* responds in captivity to food availability, food type, prey size, and hydrodynamic conditions (Da Ros et al., 2022; Gori et al., 2014; Orejas et al., 2016; Purser et al., 2010; Tsounis et al., 2010), with a marked preference for larger zooplankton such as adults *Artemia* or *Mysis relicta* (Da Ros et al., 2022). In contrast, *M. oculata*, which has smaller polyps, tends to capture smaller planktonic prey (Da Ros et al., 2022).

Beyond controlled laboratory observations, trophic markers such as stable isotopes and fatty acids have been increasingly used to investigate the feeding ecology of cold-water corals. In the Mediterranean Sea, however, such studies remain scarce. To date, only two studies have specifically investigated the diet of the primary species, *D. pertusum* and *M. oculata*, in the Mediterranean Sea, one based on bulk isotope analysis (Carlier et al., 2009) and another on total fatty acid composition (Naumann et al., 2015). Although valuable, these approaches provide only partial insights into the coral diet and nutritional condition.

Total fatty acid composition has been traditionally used to infer dietary patterns in marine invertebrates, but recent advances highlight the importance of analysing fatty acids within specific lipid classes (Couturier et al., 2020; Parzanini et al., 2023). Fatty acids, which are ubiquitously found in many lipids, serve different physiological functions such as energy storage (as neutral lipids) and maintaining membrane properties (fluidity and regulatory functions of polar phospholipids). While neutral lipids (triglycerides and wax esters) are typically preferred for trophic inferences because they are minimally modified after consumption and thus more closely reflect diet composition (Dalsgaard et al., 2003; Dodds et al., 2009), structural polar lipids (phospholipids) may selectively incorporate certain fatty acids from food or endogenous pathways (Mueller et al., 2014; Wall et al., 2024), providing valuable information on the dietary requirements of the animal (Couturier et al., 2020).

In order to better evaluate the trophic status of Mediterranean cold-water corals and address the current knowledge gaps, this study investigates the trophic ecology of *M. oculata* and *D. pertusum* using lipid class fatty acid composition to explore the following key questions:

- Are the dietary inputs sufficient to meet the metabolic needs of cold-water corals?
- What are the primary food sources for cold-water corals in the Lacaze-Duthiers canyon (NW Mediterranean Sea), and how does their diet vary locally?
- And finally, are cold-water corals in the Lacaze-Duthiers canyon in optimal trophic condition?

2. Material and methods

2.1. Study area

The Lacaze-Duthiers canyon is one of the many submarine canyons that cut the continental shelf of the northwestern Mediterranean Sea (Fig. 1). Located in the Gulf of Lion at the western end of the Pyrenean coastline, it extends across the continental slope to a depth of over 1600m and acts as a natural conduit of sediment and water to the deep-sea (Palanques et al., 2006; Ulses et al., 2008). Organic particles resuspended during episodic high energetic events such as storms and dense water cascading are exported within the submarine canyon to the mid- and lower slope and sustain rich benthic assemblages (Fiala-Medioni et al., 2012; Salvado et al., 2017; Sanchez-Vidal et al., 2008). The head of the Lacaze-Duthiers canyon is an exceptional hotspot of biodiversity and hosts the highest known densities of deep-sea corals in the western Mediterranean Sea (up to 4.3 colonies per m^2; average 2.2 colonies per m^2 over 4370 m^2, Fabri et al., 2022). Cold-water coral colonies are found on the rocky bottoms of the canyon flanks or on top of eroded boulders in sedimented areas at depths ranging from ca. 250–600m (Fabri et al., 2025). Two scleractinian species, *D. pertusum* and *M. oculata,* dominate these communities and form large three dimensional calcareous structures that provide habitat, shelter, feeding ground and breeding areas for many other species. Cold-water coral communities are acknowledged for their ecological functions and economical value, and as such the head of the Lacaze-Duthiers canyon has been included into the perimeter of the marine nature park of the Gulf of Lion since 2011 (Freiwald et al., 2004; Watremez, 2012; Würtz, 2012). Despite being part of a MPA, the corals in this canyon still face various threats, including climate change, marine litter pollution and fishing activities (Fabri et al., 2014, 2025; Durrieu de Madron et al., 2023).

2.2. Sample collection and conditioning

The sampling site (site A) is a long-term monitoring site, where coral growth and their microbiome have been monitored by the Benthic Ecogeochemistry Laboratory (LECOB, Oceanological Observatory of Banyuls-sur-mer) since 2010 as part of the long-term programme "Biodiversity, extreme marine environment and global change" (Chapron et al., 2020a, 2020b, 2021; Chemel et al., 2023; Galand et al., 2020; Lartaud et al., 2014). It is located in the Lacaze-Duthiers canyon,

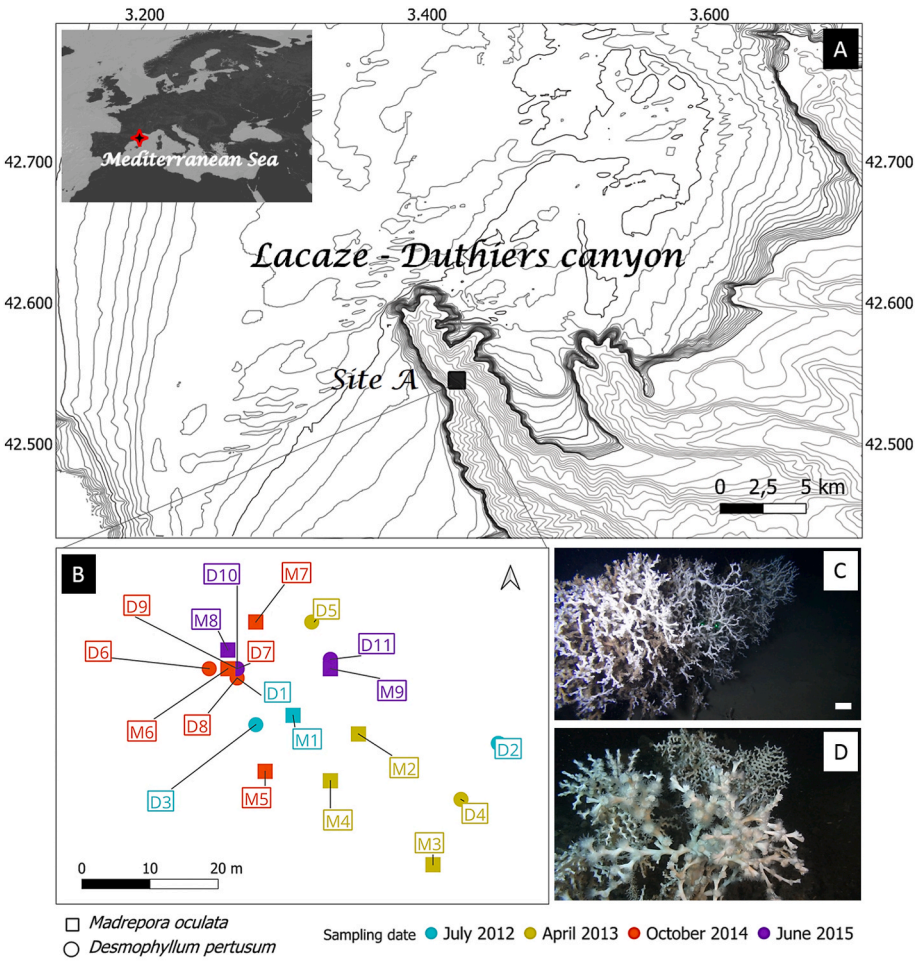

Fig. 1. (A) Location of the Lacaze-Duthiers canyon in the Gulf of Lion (NW Mediterranean Sea) with (B) a close-up on the collection area and (C–D) pictures of reefs formed by *Madrepora oculata* and *Desmophylum pertusum.*
The maps were created using the Free and Open Source QGIS. The Mediterranean map was adapted from ESRI Ocean base map. The Lacaze-Duthiers canyon map was adapted from Berné and Satra (2002).
Green laser dots on picture C are distant from 6 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

15 miles off Banyuls-sur-Mer in the Gulf of Lion at a depth of about 520m (42°32.43N, 03°25.17E) (Fig. 1). Site A is characterised by large colonies of *D. pertusum* (Linnaeus, 1758) and some smaller colonies of *M. oculata* (Linnaeus, 1758) growing on hard substrates outcropping the floor (Lartaud et al., 2017).

Branches of 11 colonies of *D. pertusum* (D1 to D11) and 9 colonies of *M. oculata* (M1 to M9) were collected during field campaigns carried out in July 2012, April 2013, October 2014, and June 2015 using the Remotely Operated Vehicle (ROV) Super Achille deployed from the R/V Minibex or the R/V Janus II of the COMEX S.A. (Lartaud et al., 2014). Some samples originated from the same coral thicket and possibly from the same colony (D1/D8 and D7/D9/D10, See Table S1 for precise location). The cold-water corals were recovered in thermally insulated polypropylene boxes (~13 °C) and conditioned immediately after recovery. The sub-apical portions of the branches were cut, flash-frozen, and stored in liquid nitrogen until return to the laboratory. Samples were then stored at −80 °C and rapidly freeze-dried to avoid lipid hydrolysis. Polyps were not separated from the carbonate skeleton as it has been demonstrated that almost half the total lipid concentration is retained in the skeleton when coral tissues are isolated using the air-spraying method and that the *in toto* crushing method should be preferred (Conlan et al., 2017). Freeze-dried coral samples were ground to a fine powder in liquid nitrogen using a TissueLyser II QIAGEN, then freeze-dried again to eliminate any residual moisture from the grinding process. Sediment cores were also collected at site A on the same date using a multitube corer (Minicorer Mark VI from Osil, Great-Britain). During subsequent field campaigns in 2017 and 2018 from the same programme, water was collected from the cold-water corals reefs and living copepods isolated on board.

2.3. Analytical procedure

Total ash was determined on ground coral samples by incineration in a muffle furnace at 400 °C for 6 h (Antao and Hassan, 2011). The ash content was subtracted from the dry weight to obtain the ash-free dry weight (AFDW), which excludes the inorganic fraction and provides the organic matter content.

Total lipids were determined from ground whole coral samples using the sulfophosphovanillin colorimetric method described by Barnes and Blackstock (1973) with minor modifications (Pruski et al., 2017). Total lipids were quantified using a calibration curve of cholesterol. Analyses were performed in triplicates with coefficients of variation below 5 %. Results are expressed in mg per gram AFDW.

For lipid class fractionation, total lipids were extracted from ground whole coral samples with a chloroform–methanol–water mixture (1v: 2v: 0.8v) according to the procedure described by Bligh and Dyer (1959), but the water used in the original protocol was replaced by a solution of sodium chloride and phosphoric acid (NaCl 1M-H_3PO_4

0.2M), which increases protein unwinding and lipid recovery. Butyl-hydroxytoluene (50 mg l^{-1}) was furthermore added to prevent oxidation during the extraction (Christie, 2003). Following partition of the monophasic solution using pure water, the organic phase was recovered whereas the aqueous phase was extracted again three times with chloroform. The organic phases were pooled, washed with a potassium carbonate/sodium hydroxide solution (K_2CO_3 2 %/NaOH 0.3N) and the solvent was evaporated in a Thermo Scientific Savant SpeedVac vacuum concentrator at room temperature. The lipid extracts were dissolved in hexane and stored at −80 °C until lipid class fractionation.

Lipid classes were fractionated by solid-phase extraction (SPE) on commercial pre-packed aminopropyl–bonded silica cartridges (strata®-NH_2 3 ml, Phenomenex®, Le Pecq, France) following Kaluzny's procedure (Kaluzny et al., 1985). The detailed protocol is available on protocols.io (Pruski and Vétion, 2025). Briefly, the lipid extracts were loaded on the cartridges preconditioned with 4 ml of hexane. Neutral lipids (NL) were then eluted with 8 ml chloroform:2-propanol (2:1, v/v), the free fatty acids (FFA) with 8 ml acetic acid in diethyl ether (2:98, v/v), and the phospholipid (PPL) fraction with 8 ml methanol. The NL fraction was dried with a SpeedVac concentrator at 45 °C and then resuspended in 200 µl of pure hexane before loading on a second preconditioned cartridge. The wax esters (WE) and steryl esters (SE) were eluted with 8 ml hexane, and a third preconditioned cartridge was placed below the second cartridge. The remaining NL were fractionated as follow: triglycerides (TG) in 12 ml diethyl ether:methylene chloride in hexane (1:10, v/v), sterols in 18 ml ethyl acetate in hexane (5:95, v/v), diglycerides (DG) in 8 ml ethyl acetate in hexane (15:85, v/v), and monoglycerides in 8 ml chloroform:methanol (2:2, v/v).

The FFA, PPL, WE/SE, TG, DG and MG fractions were evaporated with a rotary evaporator and re-dissolved in 100 µl of hexane.

Fatty acids from the 6 lipid fractions were transesterified and methylated using a solution of methanol, sulfuric acid and chloroform (1.7:0.3:2, v/v/v) for 90 min at 90 °C in the presence of butyl-hydroxytoluene to prevent lipid oxidation (see for further detailed Pruski and Vétion, 2024). Nonadecanoic acid (19:0) was added to the extraction vials as an internal standard (1 mg ml^{-1}). The organic phase containing the fatty acid methyl esters (FAME) was recovered by three successive extractions with hexane chloroform (4:1, v/v) and rinsed with a 2 % K_2CO_3 solution. An aliquot of this phase was evaporated to dryness in a SpeedVac vacuum concentrator at room temperature.

Fatty acid methyl esters (FAME) were then re-dissolved in 50 µl of hexane. The GC-MS analysis was performed on a Varian 3900 gas chromatograph coupled to a Saturn 2100 T ion trap detector equipped with a Zebron ZB-WAX (30 m length, 0.25 µm film, 0.25 mm internal diameter; Phenomenex®, Le Pecq, France) capillary column using a constant helium flow of 1 mL min^{-1}. Analytes were detected in full scan mode and quantified using external calibration as described in Pruski et al. (2022). Source assignment of individual fatty acid was based on the literature (Supplementary material, Table S2). To support these assignments, we also analysed surface sediments collected nearby and copepods isolated from the coral reefs (see Figs. S1 and S2). Total fatty acids (without prior lipid class separation) were extracted from sediments (3g dry weight) and copepods (100 individuals) by direct acid transmethylation following the protocol described by Pruski and Vétion (2024). The resulting FAME were analysed by GC-MS under the same analytical conditions as those used for the coral samples.

2.4. Statistical analyses

Due to the small sample sizes, non-parametric univariate permutation tests (function oneway_test) were employed to compare the means of organic matter (OM), total lipids, and individual fatty acids or groups of fatty acids (saturated fatty acids [SFA], monounsaturated fatty acids [MUFA], and polyunsaturated fatty acids [PUFA]) between the two species. These distribution-free tests are robust against the assumption of normality, making them appropriate for the data at hand. Non-parametric statistical analyses were used to examine relationships between variables (Spearman non-parametric test, ρ). p-values below 0.05 were considered statistically significant.

Hierarchical clustering analysis (HCA) was used to compare the fatty acid composition of the six lipid classes of the 20 samples of cold-water corals. Two fractions of PPL were not included in the analysis as they contained very low PUFA contents, which is certainly attributable to some troubleshooting during the extraction or separation steps. Fatty acids only found in trace amounts or in a few samples were removed. Percentages were then recalculated on the 26 remaining fatty acids and a square-root transformation was applied in order to reduce the weight of very dominant fatty acids (Happel et al., 2017). The fatty acid data were then standardised (divided by total margin) and a hierarchical clustering analysis (HCA) was performed using square-root-transformed Bray-Curtis dissimilarity index and Ward's aggregation method (Ward, 1963). Lipid fractions sharing a similar fatty acid composition have a high degree of similarity and cluster together. Variability in the fatty acid composition of wax/steryl esters and triglycerides among species and between sampling dates was visualised using correspondence analysis (CA), which is suitable for analysing compositional data (Greenacre and Primicerio, 2013). CA was performed on the relative fatty acid proportions (mass % of sum) and presented in the form of contribution biplots (option "rowgreen" of map argument in function fviz_ca_biplot), where the low contributing variables shrink to the centre and the high contributors stand out according to their contribution (Greenacre and Primicerio, 2013). The CA biplots enable to identify groups of samples with similar fatty acid composition, i.e. feeding in the same way. Canonical correspondence analysis (CCA) was further performed on the same datasets as a first attempt to identify the factors responsible for the observed diet patterns. The explanatory variables included physiological descriptors (species and sex/maturity), indicators of fitness (OM%, total lipids, waxes/triglycerides ratio, approximate size of colony) and date of collection (Table S1). Sex and maturity stage for these colonies have been previously reported (Chemel et al., 2023). The size of the colonies was estimated using video, based on the projection of the laser spots from the ROV with a gap of 6 cm (Fig. 1C).

All analyses were performed using R Statistical Software (v2024.4.2.764; Posit team, 2024) with the following packages: Vegan (Oksanen et al., 2016), coin (Hothorn et al., 2005), dendextend (Galili, 2015), FactoMineR (Lê et al., 2008), and factoextra (Kassambara and Mundt, 2020).

3. Results

3.1. Organic matter and total lipid content

The OM content of the coral branches was 5.2 ± 2.3 % for the colonies of *D. pertusum* and 5.7 ± 1.9 % for those of *M. oculata* (Table S1). There was a moderate, positive correlation between total lipids and OM content (Spearman rank correlation, $\rho = 0.45$). The average total lipid content in *D. pertusum* was 207.6 ± 68.6 mg g^{-1} AFDW (Table S1), the lowest value being attributable to a sample from a broken colony (D10). The average total lipid content in *M. oculata* was 244.0 ± 80.4 mg g^{-1} AFDW. No significant differences in OM and lipid contents were detected between species.

3.2. Lipid class composition

Fatty acid concentration and composition was determined in 6 lipid fractions: phospholipids (PPL), free fatty acids (FFA), esters of fatty alcohols and sterols (wax and steryl esters, WE/SE), triglycerides (TG), diglycerides (DG) and monoglycerides (MG). Most of the fatty acids were found in the storage lipids with the predominance of WE/SE over TG (~3 times more fatty acids in WE/SE than TG) and to a lesser extent associated to the membranes (PPL) (Fig. 2B). Fatty acids were also

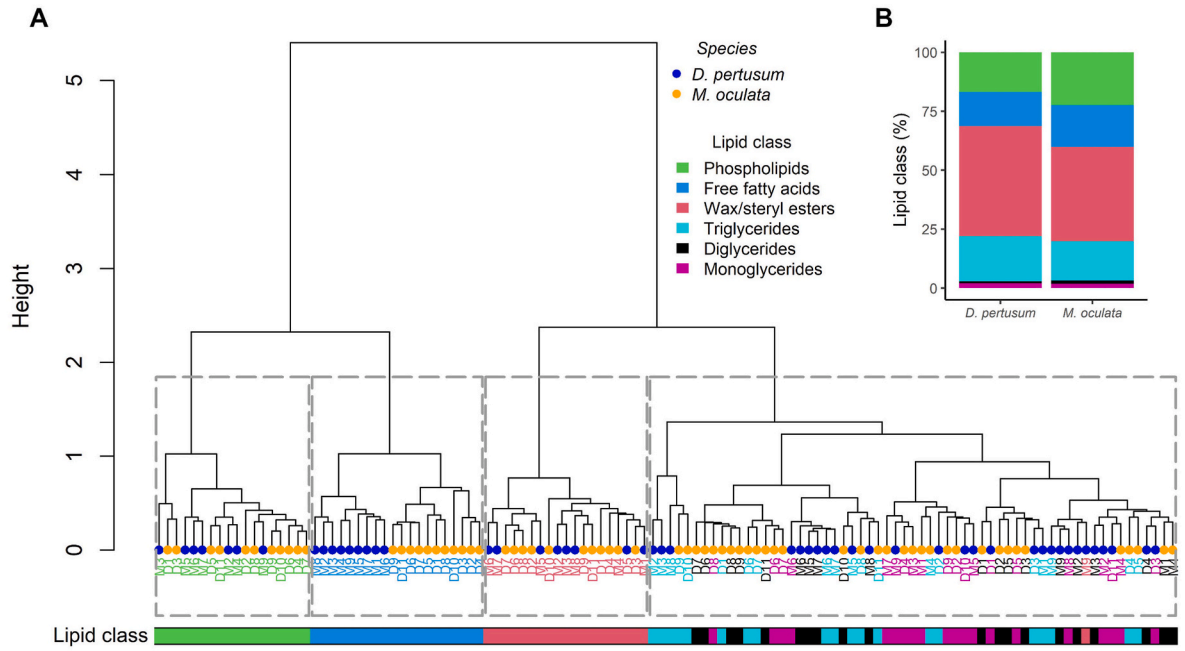

Fig. 2. Lipid class and fatty acid compositions of *D. pertusum* and *M. oculata*. (A) Hierarchical cluster analysis showing the similarity of the fatty acid profiles between lipid classes and species (n = 11 colonies of *D. pertusum* + 9 colonies *M. oculata* × 6 lipid classes/sample). Bray-Curtis dissimilarity index and Ward's minimum variance linkage method were used for clustering. (B) Barplot showing the average fatty acid distribution between lipid classes in the two species (% of total fatty acids).

present as FFA. If FFA are naturally released as intermediates of lipid metabolism, high levels are usually indicative of lipid deterioration during sample storage and/or analysis (Couturier et al., 2020). The proportion of FFA in the cold-water corals was low (2.1 ± 1.2 % of total lipids in *D. pertusum* and 1.3 ± 0.7 % of total lipids in *M. oculata*, Fig. 2B) and comparable to levels observed in other coral species (Imbs et al., 2016), an indication that sample processing did not lead to lipid autolysis. Only low amounts of fatty acids were found in the MG and DG (Fig. 2B).

The HCA based on the fatty acid profiles shows that samples aggregated according to the class with no discrimination of the two species (Fig. 2A). PPL, FFA and WE/SE formed three distinct clusters highlighting distinct fatty acid compositions, while MG, DG and TG formed one large cluster sharing a high degree of similarity. Fatty acid compositions of the 6 lipid classes are reported for all individual sampled in Table S3. Notably, in the FFA fraction, the contribution of 20:4(n-6) was

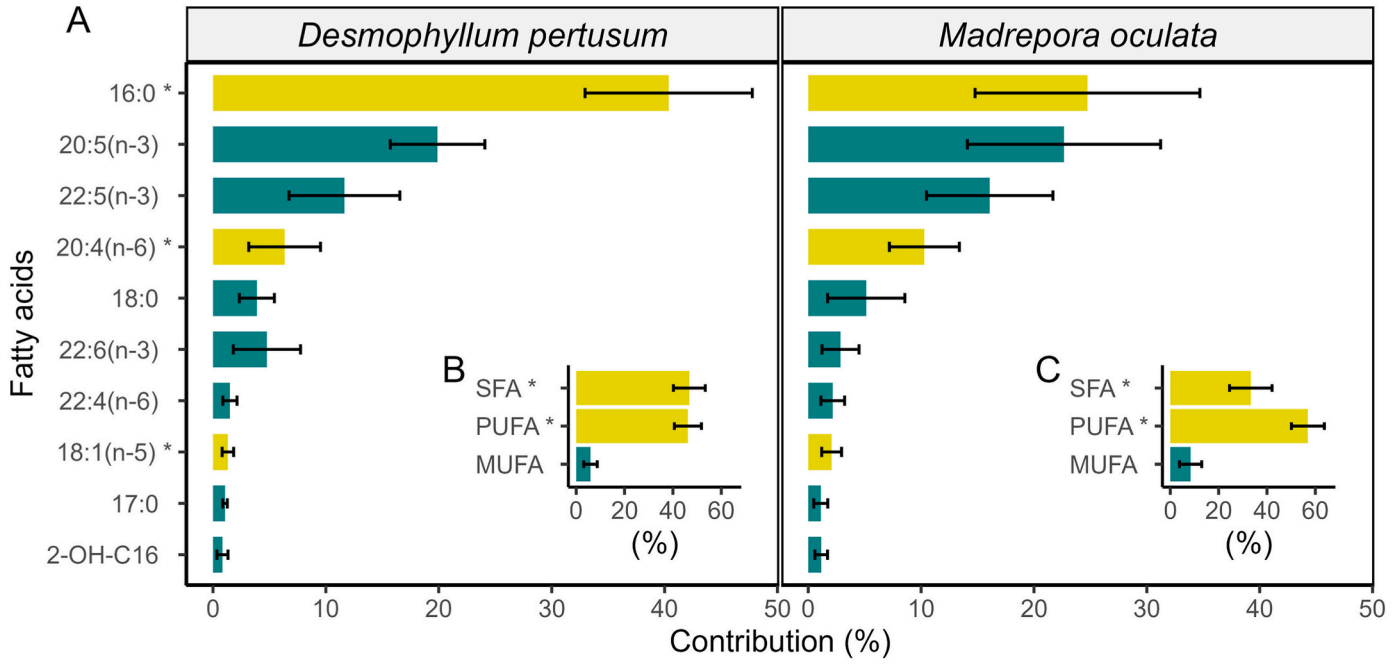

Fig. 3. Fatty acid composition of the phospholipid fraction (A) and proportion of SFA, PUFA and MUFA (B, C). Values are means and standard deviation, n = 11 for *D. pertusum* and 9 for *M. oculata*. Fatty acids contributing to more than 1 % are presented with statistically significant differences between species highlighted by lemon bars (permutation test, $p < 0.05$).

remarkably higher in *D. pertusum* (on average ~30 % of all fatty acids) compared to *M. oculata* (on average ~10 %), as shown in Table S3 (columns DW and DX, respectively). This trend was particularly striking in the broken colony D10, where 20:4(n-6) reached 57 % of the FFA fraction (Table S3, column BQ). This sample also exhibited a pronounced depletion of 16:1(n-7) and 20:5(n-3) in the FFA, WE/ST and TG fractions.

3.3. Fatty acid signature of phospholipids

A high diversity of fatty acids was found in the PPL fraction with up to 57 fatty acids identified. However, most of them were present in very low amounts (Table S3). Only 10 fatty acids contributed to more than 1 % of all fatty acids (Fig. 3A). The PPL of the two species differed remarkably by their relative proportions of PUFA and SFA (permutation test, $p < 0.005$). The PPL composition in *D. pertusum* was equally dominated by PUFA (46.2 ± 5.6 %) and SFA (46.8 ± 6.6 %), whereas MUFA were less abundant (5.9 ± 2.8 %) (Fig. 3B). In *M. oculata*, PUFA accounted for 56.9 ± 6.8 %, SFA for 33.3 ± 8.8 % and MUFA for 8.4 ± 4.6 % (Fig. 3C). In both species, SFA were dominated by 16:0 with some contribution of 18:0 (Fig. 3A), but 16:0 exhibited higher values in *D. pertusum* (permutation test, $p < 0.005$). 20:5(n-3) was the major PUFA followed by 22:5(n-3), 20:4(n-6), 22:6(n-3), and 22:4(n-6). Many fatty acids present in low amounts made up the MUFA with the predominance of 18:1(n-5). The high level of unsaturation was reflected by an average peroxidation index (PIn) of 263 in *D. pertusum* and 311 in *M. oculata* (see Table S2 for explanation on PIn calculation).

Fatty acids unique to bacteria were also found in the PPL fraction with two molecules in particular that contributed to about 1 % of the total fatty acids: the hydroxylated fatty acid 2OH-C16 and the odd number saturated fatty acid 17:0 (Table S3). Bacterial markers accounted for 2.4 ± 0.6 % and 2.9 ± 1.4 % of all fatty acids for *D. pertusum* and *M. oculata,* respectively.

3.4. Fatty acid signature of storage lipids

In both species, a high proportion of the fatty acids was found in the wax/steryl ester fraction (47 % and 43 % of the fatty acids for *D. pertusum* and *M. oculata*, respectively, Fig. 2B). The proportion of fatty acids stored as triglycerides was usually lower (19 % and 17 % for *D. pertusum* and *M. oculata*, respectively). The proportions of fatty acids stored in these two fractions presented a high inter-individual variability. As observed with the phospholipids, a high diversity of fatty acids was found in the storage lipids (on average 58 fatty acids), but only

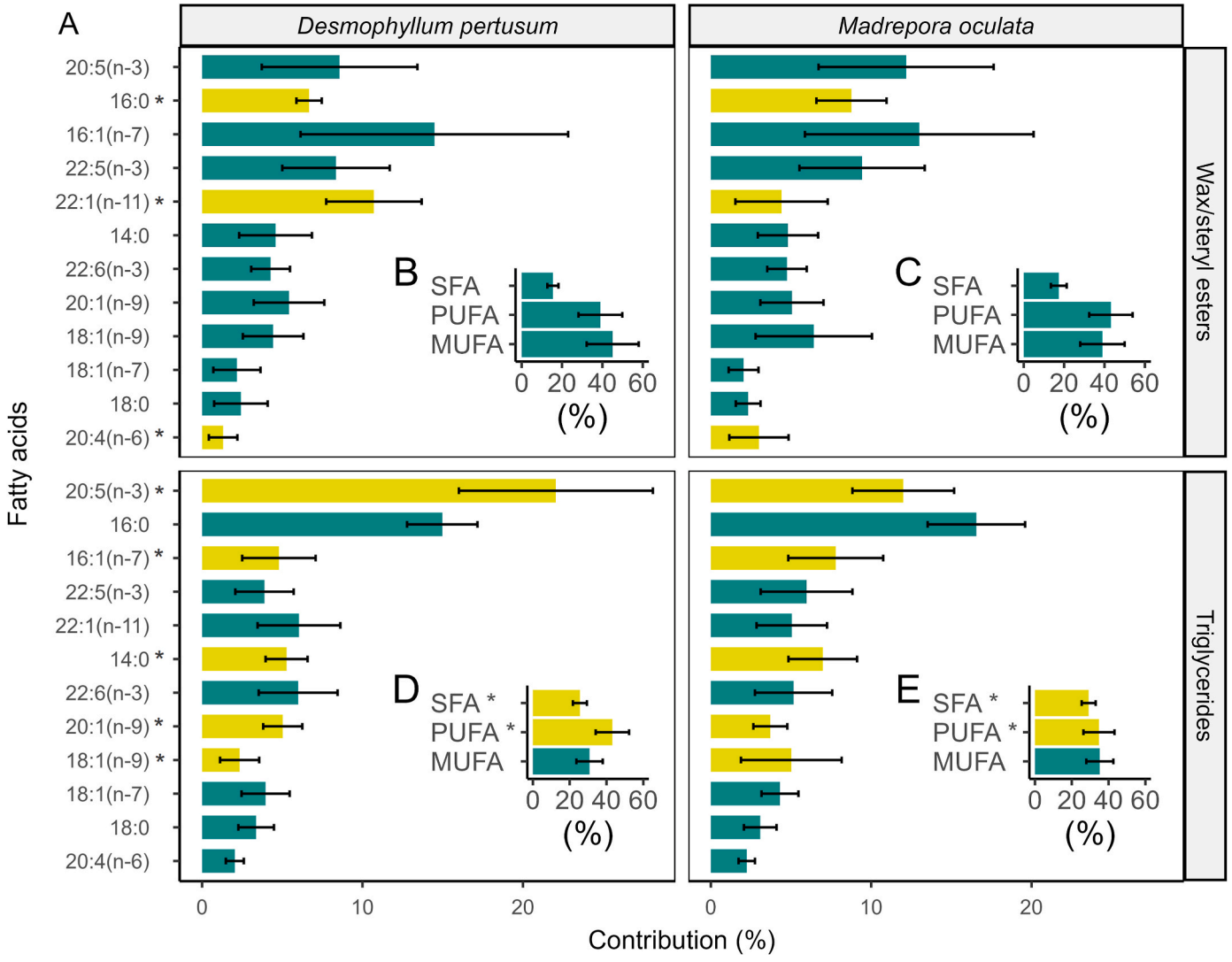

Fig. 4. Fatty acid composition of the wax/steryl ester and triglyceride fractions (A) and proportion of SFA, PUFA and MUFA (B, C, D, E) in *D. pertusum* (left panel) and *M. oculata* (right panel). Values are means and standard deviation, n = 11 for *D. pertusum* and 9 for *M. oculata*. Fatty acids contributing to more than 2 % in the two fractions are presented with statistically significant differences between species highlighted by lemon bars (permutation tests, $p < 0.05$).

17 contributed to more than 1 % of the total fatty acids (Table S3). The wax/steryl ester fraction was dominated by PUFA and MUFA (39.0 ± 10.8 % PUFA and 45.1 ± 12.9 % MUFA in *D. pertusum*, 43.2 ± 10.7 % PUFA and 39.0 ± 11.0 % MUFA in *M. oculata*, Fig. 4B and C). SFA accounted for a minor fraction (15.5 ± 2.8 % and 17.4 ± 3.9 % in *D. pertusum* and *M. oculata*, respectively) and was dominated by 16:0 (Fig. 4A). The predominant fatty acids among the wax/steryl esters were the MUFA 16:1(n-7) and 22:1(n-11), followed by the PUFA 20:5(n-3) and 22:5(n-3). The contribution of 22:1(n-11) was significantly higher in *D. pertusum*. 20:1(n-9), 18:1(n-9) and 22:6(n-3) were also important contributors.

The triglycerides were also dominated by PUFA and MUFA (Fig. 4D and E), but they contained higher proportions of SFA than the wax/steryl esters (43.2 ± 9.1 % PUFA, 30.8 ± 7.2 % MUFA and 25.6 ± 3.8 % SFA in *D. pertusum*, 34.8 ± 8.5 % PUFA, 35.2 ± 7.4 % MUFA, and 29.2 ± 3.8 % SFA in *M. oculata*). A higher average proportion of SFA was characteristic of *M. oculata* (permutation test, $p < 0.05$). The dominant fatty acids in the triglycerides of *D. pertusum* were 20:5(n-3) (22.1 ± 6.0 %) and 16:0 (15.0 ± 2.2 %). In *M. oculata*, 16:0 accounted for 16.0 ± 4.0 % of the total FA content, while 20:5(n-3) only contributed to 12.0 ± 3.2 % (significantly lower than in *D. pertusum*, permutation test, $p < 0.05$). 14:0, 16:1(n-7), 20:1(n-9), 22:1(n-11), 22:5(n-3), and 22:6(n-3) were also found in noticeable amounts in the triglycerides. The 18:1 (n-9)/18:1(n-7) ratio (Table S3) tended to be higher in wax/steryl esters than in triglycerides (permutation test, $p < 0.01$), and was significantly higher in the triglycerides of *M. oculata* (~1.16) than those of *D. pertusum* (~0.66). The 20:5(n-3)/22:6(n-3) ratio (Table S3) was similar in both fractions for *M. oculata* (~2.6), whereas it was higher in the triglycerides of *D. pertusum* (~4.3) than in the wax/steryl esters (~2) (permutation test, $p < 0.001$). Bacterial fatty acids were only present as trace amounts in storage lipids.

Correspondence analyses (CA) further enable to highlight patterns in biomarker composition among species and between sampling dates (Fig. 5). Wax/steryl esters of colonies sampled in October 2014 were characterised by higher contributions of 16:1(n-7). *D. pertusum* was furthermore enriched in 22:1(n-11) and *M. oculata* in 20:1(n-9) (Fig. 5A). Conversely, 20:5(n-3) was a higher contributor in colonies sampled in April 2013. No clear pattern can be drawn for the two other

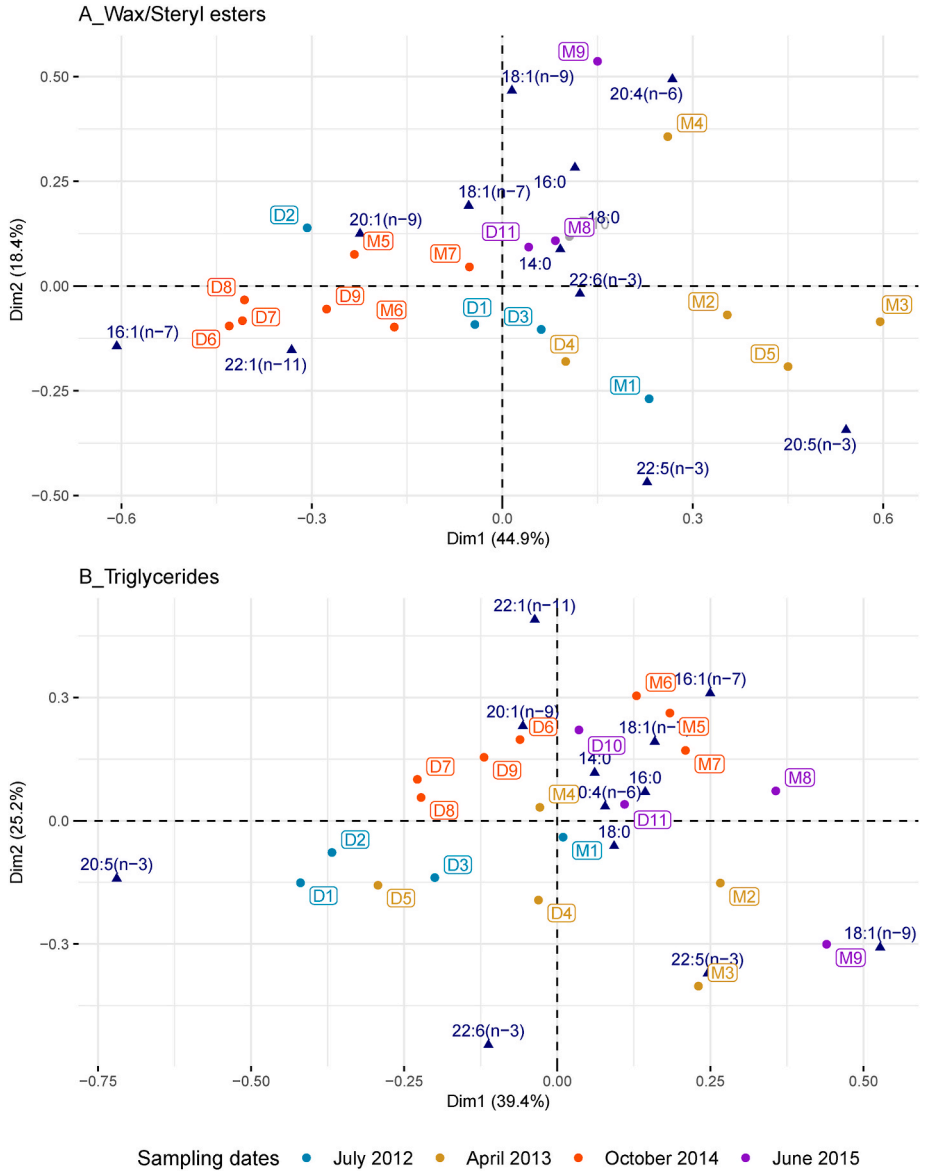

Fig. 5. Contribution biplot of correspondence analysis (CA) of fatty acids (≥2 % to the total FA content) in (A) wax/steryl esters and (B) triglycerides in *D. pertusum* and *M. oculata*. The explained variance of the 2 first axes is 63 % for wax/steryl esters and 65 % for triglycerides. Note sample D10 was placed as a supplementary row on the wax/steryl esters biplot. The two coral species are indicated by the sample labels (D stands for *D. pertusum* and M for *M. oculata*).

sampling dates, but samples of *M. oculata* exhibited higher contribution of 18:1(n-9) in June 2015. Differences between the two species were more pronounced for triglycerides than for wax/steryl esters. This difference was mainly explained by the relative contributions of 20:5(n-3) and 18:1(n-9) (Fig. 5B). Samples of *D. pertusum* (left part of the biplot) tended to be characterised by a higher contribution of 20:5(n-3), and samples of *M. oculata* (right part of the biplot) by a higher contribution of 18:1(n-9). Likewise, a clear pattern can be seen between sampling dates with samples collected in July 2012 and April 2013 (upper part of the biplot) exhibiting a higher contribution of 22:6(n-3), and those collected in October 2014 and June 2015 (lower part of the biplot) a higher contribution of 22:1(n-11) (Fig. 5B). Canonical correspondence analyses (CCA) further confirm that "species" and "date of collection" are the two factors that mostly explain fatty acid profiles of storage lipids, while size of the colony is another major factor (Table S4, Figs. S3 and S4).

4. Discussion

This study provides the first detailed comparison of fatty acid profiles across six lipid classes in two key Mediterranean cold-water coral species. Our findings confirm that phospholipids and storage lipids differ strongly in composition, reflecting their distinct functions (structural versus storage). While no species-specific fatty acid markers were identified, the data reveal differences in the trophic signature and storage strategies of *D. pertusum* and *M. oculata*, as well as significant inter-individual variability likely related to food availability, colony size, and physiological condition. Overall, both species exhibit high PUFA contents and accumulate substantial energy reserves, suggesting a good nutritional status and a strong dependence on surface-derived food sources.

4.1. Composition of cold-water coral phospholipids

Cold-water corals from the Lacaze-Duthiers canyon were characterised by a high level of unsaturation in their phospholipids, as indicated by their elevated peroxidation index (Table S3). Such high PUFA content is consistent with observations in other deep-sea organisms, and is often interpreted as an adaptation to low temperature and high hydrostatic pressure (Colombo et al., 2017), in line with the homeoviscous adaptation (HVA) theory (Hall et al., 2002; Pernet et al., 2007a, 2007b; Sanina and Kostetsky, 2002). According to this theory, membrane fluidity is maintained through adjustments in fatty acid composition, especially by increasing the proportion of unsaturated fatty acids under cold conditions. For example, the honeycomb worm *Sabellaria alveolata* shows decreased unsaturation with warming (Muir et al., 2016), while *Mytilus edulis* mussels from cold waters are enriched in ω-3 fatty acids compared to warm-water individuals (Facchini et al., 2018).

The dominance of PUFA in both species could therefore reflect an adaptive response to deep-sea conditions, as observed in other taxa such as copepods (Pond et al., 2014) and ctenophores (Winnikoff et al., 2021). However, high PUFA levels have also been reported in hydrocorals and soft corals regardless of depth (Imbs, 2013; Imbs et al., 2006, 2015, 2019), suggesting that this pattern may not be solely environmentally driven. Indeed, their glycerophospholipids typically contain a SFA at the *sn*-1 position and a PUFA at the *sn*-2 position (Imbs and Velansky, 2021). Additionally, cnidarian membranes are composed of a great variety of glycerophospholipids, whose relative abundance and fatty acid composition differ among species (Imbs et al., 2019; Imbs and Velansky, 2021). Such inherent biochemical diversity may account for the differences observed between the two coral species in our study, independently of environmental forcing (Fig. 3).

Beyond their role in maintaining membrane properties, PUFA also contribute to other key functions such as cell division (e.g. 20:5(n-3), Kawamoto et al., 2009) and endocytosis through increased membrane curvature (Pinot et al., 2014). This latter function is particularly relevant for anthozoans, where macropinocytosis (a type of endocytosis allowing the non-specific uptake of extracellular material) plays an important role in heterotrophic and autotrophic feeding (Ganot et al., 2020). Taken together, these observations suggest that the high PUFA content observed in the phospholipids of cold-water corals may not solely reflect environmental adaptation (as postulated by the HVA theory), but could also represent a conserved physiological trait supporting fundamental cellular functions such as macronutrient uptake.

4.2. Dietary requirement of membrane lipids

Our results show that phospholipids and storage lipids of cold-water corals have distinct fatty acid profiles (Fig. 2A), which is consistent with previous studies highlighting that compositional differences between these two groups of lipids are related to their respective functions (structural vs energy reserve) (Imbs et al., 2006, 2015). Phospholipids differed mostly by low amounts of MUFA in comparison to storage lipids, but all dominant fatty acids (16:0 and PUFA) were also abundant in the triglycerides and wax/steryl esters. Fatty acid composition of phospholipids is not directly affected by feeding. Yet, it reflects some requirements, in particular in PUFA for the maintenance of membrane functions. A dietary supply of PUFA is considered essential as most animals have limited capacity for *de novo* synthesis of PUFA (Monroig et al., 2013). This old paradigm has been recently questioned as many marine invertebrates, including cnidarians, possess the enzymes involved in the biosynthesis of ω3 long-chain PUFA (Kabeya et al., 2018). Recombinant yeasts expressing a desaturase from the stony coral *Acropora millipora* are able to produce 18:3(n-3), 20:5(n-3), and 22:5 (n-5) by converting ω6 precursors (Kabeya et al., 2018). They are also evidences that *D. pertusum* may produce phospholipid-derived fatty acids that are absent in its food (Mueller et al., 2014). In the boreal soft coral *Gersemia rubiformis*, the polar lipids contain high levels of 20:4 (n-6), 22:4(n-6) and 24:5(n-6), while their neutral lipids are deficient in ω6 PUFA, a good indication that these fatty acids are synthesised endogenously by octocorals (Imbs et al., 2006). This was not the case in our study where high relative proportions in 20:5(n-3), 22:5(n-3), and 22:6(n-3) were found in the storage lipids of cold-water corals as well as in their phospholipids. Although some endogenous production cannot be excluded, it clearly appears that cold-water corals from the Lacaze-Duthiers canyon can obtain a sufficient supply of ω3 PUFA from their environment. Among the PUFA belonging to the ω6 series, arachidonic acid (20:4(n-6)) was the only one that contributed significantly to the phospholipids of the cold-water corals (6 and 10 % in *D. pertusum* and *M. oculata*, respectively, Table S3). It was less abundant in the storage lipids (~1–3 %), but dominated the composition of the free fatty acids in *D. pertusum* (~30 % of the free fatty acids, Table S3). The source of 20:4(n-6) remains to be clarified. It can be biosynthesised from C_{18}-precursors in some corals (Imbs et al., 2006). However, 18:2(n-6) content was negligible in the two cold-water corals; a dietary source is thus more likely. No data are available on the composition of the phytodetritus in the Lacaze-Duthiers canyon, but 20:4(n-6) was found in the surrounding sediments (>1 % of total fatty acids, Fig. S1) confirming its occurrence in the settling particles. This result is an indication that specific dietary fatty acids are selectively assimilated in membrane lipids to meet specific functional requirements.

4.3. Fatty acid trophic markers in the Lacaze-Duthiers cold-water corals

The storage lipids of *D. pertusum* and *M. oculata* contained diverse and abundant fatty acid trophic markers (FATM), consistent with a mixed diet. When dietary intake exceeds the immediate metabolic needs of the coral, excess fatty acids are stored in intracellular lipid droplets, primarily in the form of neutral lipids such as triglycerides and wax esters (Strömberg and Östman, 2017). These storage lipids undergo minimal transformation and reflect dietary inputs over several weeks to months (Dalsgaard et al., 2003), providing an integrated signal of

previous feeding history. This temporal integration likely explains the diversity and persistence of trophic markers observed in our samples.

Among the FATM, the long chain MUFA, 20:1(n-9) and 22:1(n-11) synthesised by calanoid copepods (Dalsgaard et al., 2003), were found in significant amounts in the wax esters and triglycerides, confirming previous conclusions based on isotopic measures that herbivorous copepods represent an important part of the diet of Mediterranean cold-water corals (Carlier et al., 2009). In this regard, they do not differ from their Atlantic congeners, which rely largely on calanoid copepods. Dodds et al. (2009) found depth-related differences in the contribution of calanoid markers. They accounted for about 40 % of the total fatty acids in the wax esters of *D. pertusum* at the shallower Mingulay reef complex (130m depth) and decreased by half at the deeper sites of Rock Bank (900m depth) and New England (1312-1217m depth) indicating lower reliance on herbivorous copepods with depth. These deeper cold-water corals exhibited higher contributions in 18:1(n-9), a marker of omnivorous or carnivorous non-calanoid copepods suggesting a shift for other plankton species. In the Lacaze-Duthiers canyon, the contribution of calanoid markers to the wax esters of *D. pertusum* was similar to the values reported at Rock Bank and New England, but the omnivorous or carnivorous non-calanoid markers were remarkably lower in the Mediterranean corals implying that non-calanoid copepods only account for a minor part of their diet. There have been no studies dedicated to the description of the zooplankton community in this area, but we have identified numerous *Calanus helgolandicus* in water samples collected within the coral massifs (C. Rassoulz Pers. Obs.), an indication that corals may predate on migrating herbivorous calanoids at some periods of the year.

Another major difference between the Mediterranean and Atlantic colonies of *D. pertusum* lies in the relative PUFA content of the waxes. While Dodds et al. (2009) reported a PUFA/MUFA ratio of about 0.2, our results show much higher values (0.9) implying dietary inputs of PUFA rich wax esters. Not all species of copepods store energy in wax esters and those that do are primarily herbivorous species undergoing diapause (Lee et al., 2006). In herbivorous calanoids, the fatty acid composition of wax esters tends to reflect that of their diet, being thereby enriched in phytoplankton FATM (Pond et al., 2012). High contributions of diatom markers, eicosapentaenoic (20:5(n-3)) and palmitoleic (16:1(n-7)) acids in the wax esters of *D. pertusum* from the Lacaze-Duthiers are thus consistent with the postulate that they rely on herbivorous copepods when abundant in the environment and accumulates this lipid-rich resource for periods of food scarcity. In contrast, wax esters of *M. oculata* contained less long-chain MUFA and exhibited a higher carnivory ratio (18:1(n-9)/18:1(n-7)), which suggests a lower reliance on herbivorous copepods or a less efficient assimilation of their fatty acids. This observation is in line with the study of Naumann et al. (2015) who performed lipid biomarker analyses on *M. oculata* (total fatty acids on a pool of 10 branches) from the South Malta Coral Province (462–690 m water depth). The authors found that the non-calanoid marker, 18:1(n-9), predominated (>10 % of the total fatty acids) over the calanoid markers, 20:1(n-9) and 22:1(n-11) (~5 % each), and concluded that omnivorous and carnivorous zooplankton represented the main source of food for *M. oculata*. Our results offer a more nuanced conclusion, as the relative levels of calanoid and non-calanoid markers varied greatly among colonies (Fig. 4), highlighting that the contribution of herbivorous and carnivorous zooplankton to the diet of *M. oculata* may fluctuate, potentially in response to seasonal changes in the zooplankton community present in the vicinity of the corals.

A high diversity of PUFA was found in the storage lipids of both species, with a dominance of long chain fatty acids belonging to the omega 3 series (20:5(n-3), 20:4(n-3), 22:5(n-3), 22:6(n-3)) and lower proportions of omega 6 fatty acids (18:4(n-6), 20:4(n-6), 22:4(n-6)). These markers are usually assigned to phytoplankton and indicate a direct or indirect link with the surface productivity. Pulses of fresh phytodetritus in the Lacaze-Duthiers canyon have been reported to occur in winter and spring (i.e. increase in the amino acid and phytopigment fluxes during late-winter early spring, Buscail et al., 1990, and peak in pigments in the surface sediments at 600m depth in May–June, Riaux-Gobin et al., 2004). Yet, phytoplankton trophic markers can also be transferred to corals by the herbivorous zooplankton (i.e. the calanoid copepods, Sargent and Falk-Petersen, 1988, or other groups such as the gelatinous zooplankton, which are also enriched in PUFA, Rossi et al., 2008). Of particular interest is clupanodonic acid (22:5(n-3)), an intermediate in the synthesis of both 20:5(n-3) and 22:6 (n-3). 22:5(n-3) is a known marker of diatoms, but is also very abundant in fish oil (Dalsgaard et al., 2003). Given that phytoplankton usually do not produce wax esters, higher contributions of this marker in the waxes than in the triglycerides point to copepods being an intermediary in the transfer of 22:5(n-3).

Bacterial fatty acids were only found in trace amounts in the storage lipids of both cold-water corals, an indication that bacteria associated to detritus only account for a minor part of their diet in the field. Experimental studies have shown that *D. pertusum* can process bacteria (Mueller et al., 2014), but feeds preferentially on phytoplankton when the resource is not limiting (Van Oevelen et al., 2016). Considering the low nutritional quality of bacteria and the selective use of other, more nutritious, resources when available, it is not surprising that bacteria-derived lipids are not preserved in the storage lipids.

4.4. Drivers of dietary patterns at the local scale

The study area in the Lacaze-Duthiers canyon is a small area of about 1700 m^2 where *D. pertusum* and *M. oculata* thrive in close or mixed assemblages (Fig. 1C and D). The colonies share identical nutritional resources and environmental conditions. Yet, our investigation unveiled notable variability in the fatty acid composition of storage lipids across samples of the same species (Fig. 5). Part of this variability may stem from the positioning of branches within colonies and their alignment with main currents, which affect resource accessibility. In addition, previous studies underscored the significance of polyp position along the colony's main axis in shaping fatty acid composition in tropical corals (Conlan et al., 2018; Oku et al., 2002). To minimise this intra-colonial variability, we subsampled the branches by pooling a minimum of ~10 polyps and avoiding both the basal and apical regions, which are known to differ in age, polyp density, and metabolic activity.

Our findings indicate that colony size significantly influences the fatty acid composition of both waxes and triglycerides (Table S4). Noteworthily, larger colonies, often manifesting as robust *D. pertusum* bushes, exhibited a higher contribution of the calanoid marker, 22:1(n-11). This is in line with previous observations showing that larger colonies may alter local flow dynamics to more effectively capture zooplankton (2 cm s^{-1} Orejas et al., 2016; Sanna et al., 2023). Additionally, they can host rich benthopelagic zooplankton assemblages (authors pers. obs., Madurell et al., 2012), which may in turn contribute to their diet.

Physiological traits such as sex and maturity state may also impact storage lipid composition. Seasonal fluctuations in lipids and fatty acids in corals have been linked to reproductive cycles (Baptista et al., 2012; Lin et al., 2013; Oku et al., 2003; Pernet et al., 2002). Increased lipid levels are attributed to allocation of energy to gametogenesis and usually occur rapidly during the first stage of oogenesis or spermatogenesis, while a drop in lipids and fatty acids are commonly observed after spawning due to the loss of reproductive material (Leuzinger et al., 2003; Pernet et al., 2002; Ward, 1995). However, fatty acid content remains high in *Veretillum cynomorium* during the post-spawning period (August–October) and peaks in February (Baptista et al., 2012). The authors explained these trends by high temperatures and early onset of the first stage of oogenesis in the summer, whereas during the prevernal season the increase of food triggers the final stage of gametogenesis and a higher allocation of energy to egg development. Changes in lipid composition during oogenesis have also been reported, including a reduction in wax esters in late-stage oocytes of gorgonians, potentially

linked to organogenesis (Lin et al., 2013). In *D. pertusum*, a significant decrease in both neutral and polar lipid-derived fatty acids was observed between October and February, consistent with the spawning period and substantial parental reproductive investment (Maier et al., 2020). In our study, male and female colonies were characterised at distinct maturity stage with immature polyps in about 25 % of the samples and fully mature polyps in others (e.g. eggs reaching 3–4 μm, Table S1). While lipid accumulation in eggs during oogenesis undoubtedly affects female coenenchyme composition, a link with lipid content or fatty acid composition could not be seen in the present study (Table S4). This lack of effect may be attributed to the limited sample size and collection dates, which occurred too far from *D. pertusum* spawning period, allowing for coral recovery (i.e. spawning starts in fall in the Lacaze-Duthiers canyon, Chemel et al., 2023). Continuous reproduction in *M. oculata* further complicates targeting the post-spawning period.

As previously noted, interspecific differences in fatty acid profiles were observed between the two species, likely attributable to variations in feeding preferences and/or the metabolic pathways involved in the conversion of dietary fatty acids into endogenous compounds. However, despite these differences, no species-specific trophic biomarkers were identified, indicating significant degree of dietary overlap and a strong reliance on mesozooplankton. Sampling time emerged as a key factor influencing fatty acid composition in storage lipids. Considering the corals' apparent lack of food limitation, it is reasonable to expect their storage lipid fatty acid composition, particularly wax esters, to closely mirror that of available trophic resources, including living preys and planktonic detritus exported from surface waters (Wall et al., 2024). We thus pooled results from both species to get an overview of the dynamic of the trophic resources available in the environment at the different dates. The overall contribution of the calanoid markers, 20:1(n-9) and 22:1(n-11), progressively increased in the waxes from April to July and remained high in October, implying that overwintering calanoid copepods are a long lasting resource in the Lacaze-Duthiers canyon (Table S5). Their oil sac rich in lipids enable the corals to build up reserves to withstand period of food scarcity. Conversely, the marker of carnivorous copepods, 18:1(n-9) peaked in the triglycerides in June and in the waxes in October, suggesting a more punctual resource. Note that both herbivory and carnivory FATM have been identified in copepods isolated from the study area in summer, an indication that these two types of preys might co-occur (Fig. S2). Lastly, a shift from high 20:5 (n-3) in spring to higher 16:1(n-7) in autumn was observed for the waxes (Fig. 5 and Table S5). Although both fatty acids serve as indicators for diatoms, with the latter also being characteristic of bacterial presence, our findings highlight a notable shift in the nutritional composition of the resources accessible to the corals. The Lacaze-Duthiers canyon typically experiences an influx of high quality particulate organic matter from late winter to early spring (also reflected in the higher contributions of 22:6(n-3) and 22:5(n-3) in the triglycerides), attributable to increased surface water productivity and meteorological conditions that favour the advection by dense water cascading of freshly deposited material from the shelf (Buscail et al., 1990; Fabres et al., 2008). Hence, the observed pattern between 20:5(n-3) and 16:1(n-7) suggests a higher reliance on freshly produced surface water resources in spring, contrasting with a greater dependence on organic matter recycling within coral reef ecosystems during stratified summer-fall months. While our findings shed light on significant seasonal fluctuations in coral feeding dynamics, further investigations are imperative to refine our comprehension of resource availability timing.

4.5. Trophic status of cold-water corals in the Lacaze-Duthiers canyon

The generally favourable conditions observed in the Lacaze-Duthiers canyon, such as regular pulse of food inputs and suitable hydrodynamic regimes, have long supported the development of dense and structurally complex cold-water coral communities. While these features suggest a potentially good ecological status, assessing coral health remains challenging, particularly in the deep-sea. Our results offer new insight into this question by complementing physiological observations with biochemical markers.

In this context, tissue energy reserves, here assessed through lipid and fatty acid profiles, can serve as valuable indicators of the balance between food availability and metabolic costs, reflecting the coral's ability to sustain itself in its environment (Beck et al., 2024; Wall et al., 2024). Previous experimental studies have shown that food supply significantly influences coral fitness, including growth, respiration, microbiome composition, and stress resistance (Beck et al., 2023; Büscher et al., 2017; Galand et al., 2020; Maier et al., 2020; Van Oevelen et al., 2016). However, responses vary by species: while *D. pertusum* can maintain basic metabolic functions during short-term food limitation (Maier et al., 2019), long-term starvation (6 months) leads to reduced respiration (Baussant et al., 2017; Larsson et al., 2013b). In contrast, *M. oculata* shows signs of physiological stress after just four weeks without zooplankton (Naumann et al., 2011). A marked depletion in lipid stores has also been associated with reduced food availability in both species, supporting the use of tissue reserves as sensitive trophic indicators (Galand et al., 2020; Larsson et al., 2013a; Maier et al., 2019; Naumann et al., 2011).

Our findings provide indications that the trophic status of cold-water corals in the Lacaze-Duthiers canyon is apparently good. Their tissue and total lipid contents were similar to or exceeded the values reported for *D. pertusum* and *Desmophyllum dianthus* in the Atlantic (Dodds, 2007; Kutti et al., 2022; Larsson et al., 2013a; Wall et al., 2024). Additionally, they contained high levels of storage fatty acids, primarily in the form of long-term reserves (wax esters) enriched in PUFA. These results suggest that the local environment provides high quality food in sufficient quantity to sustain the dietary requirements of the corals and withstand period of food limitation.

These biochemical findings align with *in situ* observations of healthy-looking colonies in the Lacaze-Duthiers canyon (Fabri et al., 2022), and with growth rates reported in the area, which are comparable to or higher than those measured in the North Sea and the Gulf of Mexico (Chapron et al., 2020b; Lartaud et al., 2014, 2017). Chapron et al. (2000b) further showed that coral growth and budding rates vary significantly depending on the hydrological regimes: (1) high cascading events and/or NE storms induce strong shelf erosion and organic matter remobilisation, enhancing polyp development, (2) in contrast, years with no cascading lead to sedimentation and low or no growth, (3) moderate winter down-welling events result in exceptionally high growth rates. Extrapolation of these observations would however deserve more replications to support inferences on causality.

During our study period, optimal conditions (type 3) were not observed; 2012 and 2013 were characterised by intense cascading, whereas 2014 and 2015 lacked winter down-welling. Yet, no major differences in the lipid composition were detected between these contrasting periods. This could be due to confounding factors of variability and limited replication. Still, our findings show that even under non-optimal hydrodynamic regimes the availability of high quality food was sufficient to allow cold-water corals in the Lacaze-Duthiers canyon to build and maintain energy reserves.

Looking ahead, the predicted decline in cascading events in the near future (Herrmann et al., 2008) could limit food accessibility in the canyon. This may particularly affect younger or smaller colonies, which are likely more sensitive to sediment coverage and food limitation.

Finally, sample D10, collected from a broken colony (~8 months before sampling), provides some clues on the metabolic response of corals under stress. Compared to neighbouring colonies D7 and D9, D10 exhibited lower total lipids and reduced wax/steryl esters and triglycerides. Fatty acid profiles revealed a strong decrease in 16:1(n-7) and 20:5(n-3) in most fractions and a streaking increase in 20:4(n-6), a known precursor in the stress response, in the free fatty acid fraction (Table S3). This pattern suggests that the broken colony had mobilised and relocated its storage fatty acids to meet energetic demands while

preserving membrane integrity.

Although extrapolation from laboratory to field is difficult, our observations are consistent with experimental results: in feeding trials, *D. pertusum* subjected to long-term starvation showed lower organic content, but only moderate declines in storage fatty acids, and no clear effect on skeletal growth (Baussant et al., 2017; Larsson et al., 2013a). In the case of D10, however, the signs of degradation appear more pronounced, likely due to the combined effects of mechanical damage, tissue loss, sediment exposure, and reduced access to food.

These observations underline the vulnerability of cold-water corals in the Lacaze-Duthiers canyon to physical disturbances such as longlines and nets. Despite the protected status of the area, these fishing practices are still permitted and represent a significant threat to the health and persistence of local coral populations.

4.6. Conclusions

The application of fatty acid trophic markers across lipid classes has yielded significant insights into the natural feeding behaviours and dietary requirements of two key Mediterranean cold-water coral species. The Lacaze-Duthiers canyon provides sufficient food quality and availability to supply essential fatty acids, enabling these corals to fulfil their metabolic requirements and endure periods of food scarcity. Despite variations in their specific feeding preferences, both species exhibit a strong dependence on surface productivity and migrating zooplankton. This reliance places them at considerable risk from ongoing environmental changes that could disrupt the efficiency of trophic transfer through the water column, potentially impacting their survival and ecological roles within the canyon ecosystem.

CRediT authorship contribution statement

Audrey M. Pruski: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gilles Vétion:** Writing – review & editing, Methodology, Formal analysis. **Franck Lartaud:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Erwan Peru:** Writing – review & editing, Visualization, Data curation. **Nadine Le Bris:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dsr.2025.104573.

Data availability

Data will be made available on request.

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