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REPORT

Main lipid classes in some species of deep-sea corals in the Newfoundland and Labrador region (Northwest Atlantic Ocean)

D. Hamoutene · T. Puestow · J. Miller-Banoub · V. Wareham

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Abstract Corals contain large quantities of lipids in their tissues; these lipids may be either structural or for storage. Little information is available about the lipid content of deep-sea corals, as well as ratios of main lipid classes. In this study, lipid percentages of 81 deep-sea specimens were measured and the presence of six major classes, including sterols (STEROLS), free fatty acids (FFA), triacylglycerols (TG), monoalkyldiacyl glycerol (MADAG), wax (WAX), and sterol esters (SE), was assessed. Deep-sea corals had fewer lipids than their shallow water counterparts. Decision-tree analysis revealed a link between coral groups and total lipid percentages, showing that species within the same group were characterized by similar lipid amounts. Depth did not seem to impact the total lipid percentages, suggesting that deep-sea corals adapt to the differential access to food by changing the proportion of lipid classes while maintaining equivalent lipid levels. In deep-sea species, similar to their shallow water counterparts, energy seems to be stored as neutral lipids (wax esters and triacylglycerols), with the notable difference that a high proportion of MADAG is present. These compounds are less rich in energy than TG. Depth trends were found for FFA, TG and SE with an increase in percentages after 800 m suggesting a potential need for storage due to decreased food availability. A subsequent decrease after 1,100 m was observed for FFA and TG but a more detailed investigation is warranted as the number of specimens acquired from these depths was less than 20. It is nonetheless a surprising result as increased storage is expected when food sources are sparse.

Keywords Deep-sea corals · Lipids · Storage lipids · Structural lipids

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Introduction

Deep-sea corals are recognized as important, if not crucial, parts of deep marine ecosystems (Mortensen et al. 1995; Freiwald and Roberts 2005). They provide important habitat for a variety of fish species, including some commercial fish species (Jensen and Frederiksen 1992; Buhl-Mortensen and Mortensen 2004; Wareham and Edinger 2007). Little information is available on the general physiology and biochemistry of deep-sea corals, and numerous questions remain unanswered.

Corals contain large quantities of lipids in their tissues. These lipids may be either structural or for storage (Patton et al. 1977; Stimson 1987). They are distributed throughout coral tissues and associated with cells and eggs (Stimson 1987). To date, most studies on lipid composition concern shallow water corals. These studies show that the percentage of lipids in shallow reef corals ranged from 6 to 47% of their tissue dry weight (Harland et al. 1993; Grottoli et al. 2004). Lipid levels in coral tissue appear to vary with the energy balance between input from zooxanthellae and output as respiration, cell renewal and release of reproductive materials (Battey and Patton 1984; Ward 1995). Changes in the amounts of the lipids present in coral tissues can be an indicator of stress. For example, amounts of lipids in corals

decreased during the El Niño warming event of 1983 (Glynn et al. 1985). Yamashiro et al. (2001) also found a significant difference in composition and levels of lipids between normal coral tissue and tumor tissue. In addition to producing a change in total lipids, environmental conditions could have an impact on lipid allocation: reproductive lipid versus storage lipid (Ward 1991). Lipid amounts can give insights into coral physiology and yield information on species general health status.

In this study, lipid percentages of 81 specimens were measured and the presence and ratios of six major classes of lipids were assessed. Nine deep-sea coral species were represented. This study provides novel information on the amounts of lipids in deep corals to help to understand lipid allocation and distribution in these species.

Materials and methods

Coral samples

Coral samples were gathered opportunistically from three sources: the Canadian Department of Fisheries and Oceans (DFO) multispecies stock assessment surveys, the northern shrimp stock assessment survey (DFO and Northern Shrimp Research Foundation), and the Fisheries Observer Program (FOP). All specimens were assigned a species code, bagged with locator number, and frozen. Samples used in this study were collected in the fall of 2004 and 2005 (Wareham and Edinger 2007). The species collected belonged to three orders: Alcyonacea, Antipatharia and Pennatulacea. Samples were selected to ensure a sufficient representative number of specimens to provide information on potential depth profiles. Sampling depths varied between 50 and 1,500 m but not all species were represented at all depths. Sampling sites comprised Cape Chidley (60°N, 62°W), southwest (44°N, 50°W) and southeast Grand Bank (42°N, 48°W).

The coral samples belonged to the order Alcyonacea which is subdivided into three informal groups; soft corals with polyps contained in massive bodies, gorgonians with a consolidated axis, and gorgonians without a consolidated axis (Bayer 1981). Soft corals consisted of one alcyoniid [*Anthomastus grandiflorus* (Ag), Verrill 1878] and two nephtheids [*Gersemia rubiformis* (Gr), Ehrenberg 1834 and *Capnella florida* (Cf), Verrill 1869]. Four species of gorgonians with a consolidated axis were also considered for this study: *Acanella arbuscula* (Aa) (Johnson 1862) *Paramuricea* spp. (Ps) (*P. grandis*, Verrill 1883, *P. placomus*, L.), and *Primnoa resedaeformis* (Pr) (Gunnerus 1763). One species of gorgonian without axis was considered: *Paragorgia arborea* (Pa) (L.). The order Antipatharia was represented by *Bathypathes* spp. (Bs) (Brooke 1889). Further identification at the species level was difficult. The order Pennatulacea was represented by two species *Anthoptilum grandiflorum* (Verrill 1879), and *Pennatula grandis* (Ehrenberg 1834) and designated by the common name, sea pen (Ss), because of identification difficulties.

Lipid extraction and analysis

Frozen samples were processed directly with no thawing by cutting a few polyps (0.5–2 g of tissue). Samples were rinsed under tap water, and dried for 24 h at 55°C (Ward 1995); 1 g of dry tissue was then placed in 15 ml of chloroform-methanol (CM 2:1 by vol) at room temperature for 1 day to extract lipids (Harriott 1993). After 24 h in the fume hood, samples were placed in glass tubes and centrifuged for 1 min to get rid of debris. The supernatants were transferred to glass tubes, and dried under a nitrogen stream (40–50°C). The crude lipid fraction was weighed and carefully redissolved in CM to a concentration of 1 mg ml^{-1}. Lipids were redistributed in four vials for freezing. Samples with more than 1 g of tissue were extracted twice by using two groups of polyps (tips). The reproducibility of the lipid extraction process was evaluated by calculating a coefficient of variation between replicates for a subset of samples ($n = 24$).

To assess the effect of freezing, lipid extraction was carried out on frozen as well as fresh samples. The fresh samples were kept in seawater aquaria at the Ocean Sciences Center (Memorial University of Newfoundland). One *Gersemia* colony was used and divided into six groups of polyps, three of them were frozen while three others were analyzed immediately. It is important to note that it is likely that the method used in this study extracts not only lipids but also other cellular extracts (Harriott 1993). All extracts will be referred to as lipids throughout this document.

For analysis of total lipid composition, aliquots of lipid extracts (40 µg = 40 µl) were applied to 10 × 10 cm HPTLC plates. The plates were first developed to their full length with hexane and successively benzene, and finally to half-length with hexane/ether/acetic acid (70:30:1, by vol). After drying in a stream of air, the plates were immersed in phosphoric acid/33% acetic acid/sulfuric acid/0.5% copper sulfate (5:5:0.5: 90, by vol) for 40 s and heated at 110–120°C for 24 or 48 h (Brod et al. 1991). The chromatograms were scanned by an image scanner with a gray scale mode. Percent composition of the lipids was determined on the basis of band intensity (Yamashiro et al. 2001). Image analysis was performed using GEOMATICA software. The chromatograms were digitally scanned and converted to 8-bit grey level intensity images. Individual masks were generated for each exposed area by means of on-screen digitizing. The area under each mask was calculated (expressed in number of pixels), and the intensity values

under each mask were extracted and subsequently multiplied with the corresponding number of pixels to yield intensity-area values. The same procedure was applied to unexposed areas in the standards lanes to obtain an estimate of the background exposure on each chromatogram. This background estimate was subtracted from the corresponding intensities obtained from exposed areas.

The spot intensity by this method was found to be linear up to 5 μg for all standard lipids. The classes of lipids identified on the plates were sterols (STEROLS, standard lipid = cholesterol), free fatty acid (FFA, standard = oleic acid), triacylglycerol (TG, standard = glycerol trioleate), monoalkyldiacyl glycerol (MADAG, standard = 1-o-hexadecyl-2,3-dihexadecanoyl-rac-glycerol), wax (WAX, standard = stearyl oleate) and sterol esters (SE, standard = cholesterol oleate). Results are expressed as percentages of the total lipid content for each lipid class.

Statistical analysis

Decision tree analysis (DTA) was carried out to explore trends and differences in lipid composition resulting from variations in water depth, sampling site location and species. DTA allows the formulation of relationships between one response (i.e., dependent) variable and several predictor (i.e., independent) variables by dividing a data set recursively into smaller, increasingly homogeneous portions. The final result constitutes a division of the original data set into mutually exclusive and exhaustive sub-sets (Morgan and Sonquist 1963; Kass 1980; Hawkins and Kass 1982; Breiman et al. 1984; Quinlan et al. 1987; Biggs et al. 1991; Safavian and Langrebe 1991).

Performed for either exploratory analysis or predictive modelling, DTA requires no limiting assumptions about data distributions and independence of predictor variables, allows the simultaneous handling of categorical and continuous variables in the same data set, and permits the detection of non-linear interactions between variables (Lees and Ritmann 1991; Fabricius and Coetzee 1992; Dymond and Luckman 1994; Amrhein et al. 1999; Costanza and Paccaud 2004; Chang and Chen 2005; Crall et al. 2006). These properties make DTA ideally suited to problems with limited or no a-priori knowledge about the distribution and interaction of parameters in question.

DTA was carried out using the procedure described by Breiman et al. (1984). At every level of the tree, stepwise splitting is performed by examining each of the predictor variables in turn and selecting the predictor resulting in the smallest within-group sum-of-squares for a binary split. The splitting criterion is expressed as proportional reduction in error (PRE), with a minimum PRE of 0.05 required for a split to result for any given predictor variable. The procedure supports both continuous and categorical variables. Categorical predictor variables used in the analysis include sampling site location ("*Location*") and coral species ("*Species*"). Water depth measured in meters ("*Depth*") was included as continuous predictor variable. The risk of overfitting was controlled by specifying a minimum number of cases, or stop size, for the creation of new nodes (Puestow et al. 2001). That is, if a given node contained fewer observations than the specified stop size it was not further partitioned. A stop size of 5 was selected for all tree models. DTA was applied to the entire dataset to examine the relationship between the predictor variables and total lipid composition ("Total Lipids") expressed in percent. DTA was also applied to each of the individual lipid classes ("FFA", "MADAG", "SE", "STEROLS", "TG" and "WAX"), also expressed in percent. In the case of individual lipid classes, the predictors were used in concert as well as separately to investigate the impact of each independent variable on each class of lipids. One way ANOVA was subsequently applied to all terminal nodes to reveal statistically significant differences between all subsets. The Holm-Sidak method was used for multiple comparisons.

All data were transformed into Arcsin square root prior to statistical analysis but this transformation showed no effect on decision tree analysis. Arcsin square root transformation is necessary when a sizeable number of the observed proportions are either relatively small ($P < 0.2$) or large ($0.8 < P < 1$); if most of the computed proportions lie between 0.2 and 0.7, it should have little impact on the results (Snedecor and Cochran 1980).

Results

Total lipids

Lipid analyses were carried out on two groups of polyps sampled as replicates (11 samples with less than 1 g of polyp tissue were extracted only once). The coefficient of variation (CV) between replicates (calculated for $n = 24$) varied between 9.69 and 38.39% with a mean CV value of 10.22%. Preliminary testing of *Gersemia* polyps showed no effect of freezing on lipid percentages (t test, $P = 0.758$). Similarly, no changes in percentages of the six lipid classes were found between frozen and fresh samples.

The total lipid content for all species varied between 2.43 and 38.80% with a mean of $12.2 \pm 7.73\%$ (mean $\pm$ SD). The gorgonian corals (Aa, Ps, Pr, Pa) had the lowest mean lipid content (6.53%) with values varying between 3.92 and 8.53%, while the Antipatharia (Bs) had the highest values with 27.6% (Table 1). Decision tree analysis applied to total lipid content, identified *Species* as the most important variable explaining variation in lipid content, and showed no effect of *Depth* or *Location* (Fig. 1a).

Table 1 Lipid composition and depth distribution of deep-sea coral species

Species	Ag ($n = 7$)	Gr ($n = 22$)	Cf ($n = 16$)	Aa ($n = 11$)	Ps ($n = 3$)	Pr ($n = 10$)	Pa ($n = 3$)	Bs ($n = 5$)	Ss ($n = 4$)	Mean
Lipids										
STEROLS	9.19 ± 3.66	10.40 ± 5.41	9.37 ± 4.52	12.80 ± 6.40	14.60 ± 1.99	11.30 ± 2.58	10.70 ± 2.62	9.83 ± 2.45	17.00 ± 7.53	10.90 ± 5.01
FFA	9.44 ± 4.62	10.40 ± 3.50	9.81 ± 4.28	14.90 ± 4.67	14.80 ± 3.12	11.70 ± 3.67	8.53 ± 7.10	11.70 ± 4.07	12.30 ± 3.85	11.30 ± 4.27
TG	8.14 ± 5.14	5.59 ± 3.38	10.40 ± 5.11	6.12 ± 3.97	3.94 ± 1.57	6.82 ± 4.21	8.91 ± 7.64	12.30 ± 6.38	10.30 ± 8.62	7.71 ± 5.08
MADAG	13.70 ± 8.20	15.10 ± 17.3	19.70 ± 10.10	11.40 ± 6.08	12.20 ± 2.21	12.00 ± 4.32	10.60 ± 0.94	15.40 ± 6.03	8.63 ± 2.70	14.40 ± 11.00
WAX	12.10 ± 6.91	14.60 ± 8.69	14.50 ± 7.92	10.10 ± 5.43	12.30 ± 11.40	15.00 ± 6.70	9.97 ± 6.83	14.60 ± 9.01	21.70 ± 7.18	14.00 ± 7.78
SE	6.26 ± 4.49	3.51 ± 1.89	5.25 ± 4.00	4.17 ± 3.54	13.20 ± 8.18	8.26 ± 3.90	16.80 ± 1.46	11.10 ± 5.59	4.95 ± 1.64	5.87 ± 4.56
OTHERS	41.17	40.40	30.97	40.51	28.96	34.92	34.49	25.07	25.12	33.51 ± 6.39
Total lipids	10.50 ± 6.18	12.10 ± 4.81	16.00 ± 7.16	6.93 ± 5.58	3.92 ± 1.38	6.47 ± 3.50	8.53 ± 4.14	27.60 ± 9.42	16.20 ± 2.18	12.20 ± 7.73
Depth (m)	578–1277 m	51–1135 m	59–1302 m	296–1154 m	644–1193 m	162–1157 m	448–1277 m	876–1287 m	663–969 m	

All lipid data is expressed as mean percentage ± standard deviation. Depths are expressed in meters: minimum depth–maximum depth

Ag Anthomastus grandiflorus, *Gr* Gersemia rubiformis, *Cf* Capnella florida, *Aa* Acanella arbuscula, *Ps* Paramuricea spp., *Pr* Primnoa resedaeformis, *Pa* Paragorgia arborea, *Bs* Bathypates spp., and *Ss* sea pens

One-way ANOVA applied at each terminal node confirmed the results obtained with DTA (Table 2). Figure 2 also illustrates the fact that depth is not a direct factor in determining lipid content; for example, deep species like Bs have high lipid percentages (27.60%) while Ps (3.92%), Pa (8.53%), Ag (10.50%), or Ss (16.20%) also located at depths >400 m have low (Ps,Pa,Ag) or intermediate (Ss) lipid levels. It is interesting to note that coral species belonging to the same group had percentages in a similar range. Lipid percentages increased from gorgonians to soft corals/sea pens (grouped together in terminal node) to the antipatharians.

Lipid composition

Among the lipid classes investigated (when combining all species), the lipid class with the highest proportion was MADAG (14.40%) followed by WAX (14.00%) and FFA (11.30%). The SE represented the lowest proportion of lipids extracted. The composition of the remaining 33.51% of lipids not included in this analysis is unknown; they are designated as OTHERS in Table 1. These lipids may have varying composition. Some unidentified grey areas were observed between FFA and TG on 29 plates of the 70 plates analyzed. The remaining 41 HPTLC plates showed that the unknown lipids constitute polar lipids located at the bottom of the plate.

Most of the samples were acquired at Cape Chidley ($n = 33$ from a total of 81) and southwest Grand Bank ($n = 46$). DTA showed that *Location* was not an important predictor of lipid composition. The variables *Depth* and *Species* were not entirely independent from one another. Figure 2 shows that species are distributed unequally across depths with some of them present only at depths exceeding 500 m, while others (soft corals and Pr) occur at depths from 100 to 1,000 m.

When three predictors were used in concert, most lipids (except MADAG- see comments below) showed a strong effect of the variable *Species* (Figs. 1, 4). It was therefore chosen to present to the reader the application of the *Depth* variable only when investigating effects of predictors separately (Fig. 3). The *Species* effect being well captured by the three predictors' tree. Subsequent one-way ANOVA confirmed that in 62% of the cases differences between terminal nodes were statistically significant (Table 2).

DTA confirmed with ANOVA, for STEROLS showed differences between species with Aa, Ps, and sea pens having higher values than the other species (Fig. 1b; Table 2). When using only *Depth*, a decrease of STEROLS was observed after 1,200 m (Fig. 3a).

FFA results showed differences between species with no effect of depth on Aa and Ps and higher levels of FFA for both these species (Fig. 1c; Table 2). The remaining

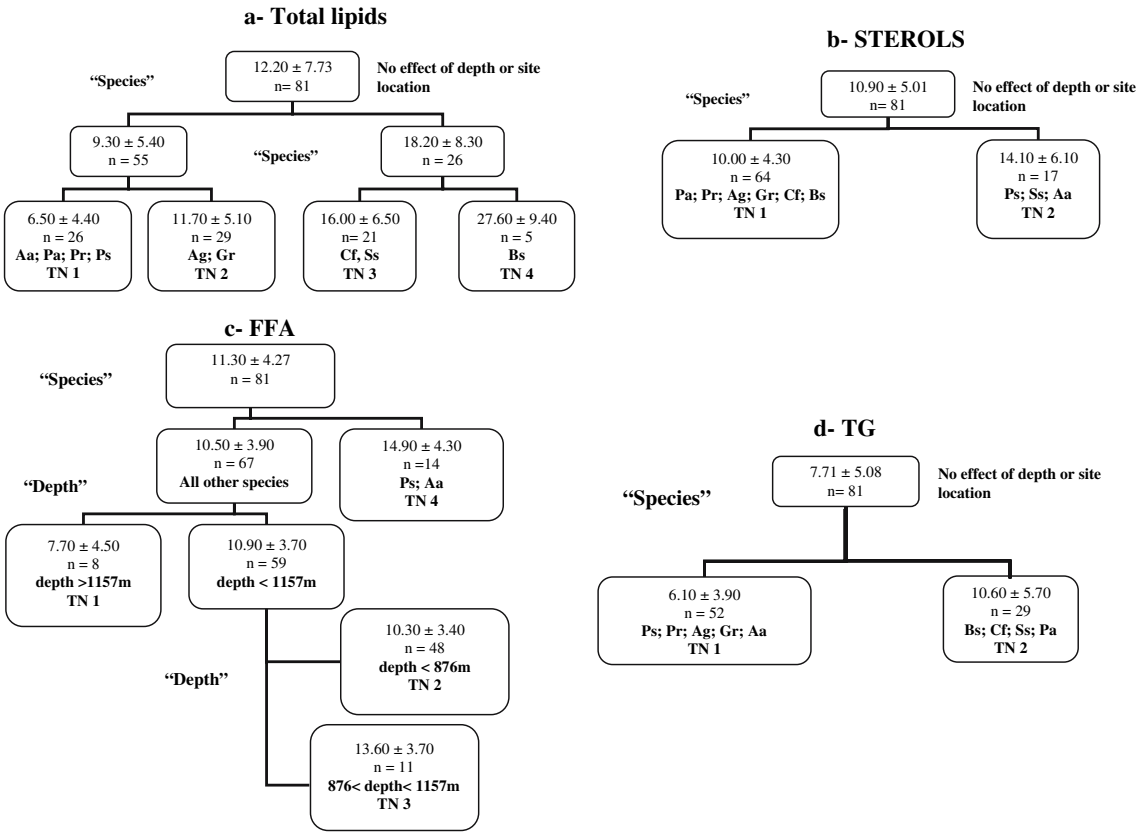

Fig. 1 Decision tree analysis for **a** *total lipids*, **b** *STEROLS*, **c** *FFA*, and **d** *TG* with three predictors: *Depth*, *Species* and *Location*. *Ag Anthomastus grandiflorus*, *Gr Gersemia rubiformis*, *Cf Capnella florida*, *Aa Acanella arbuscula*, *Ps Paramuricea* spp., *Pr Primnoa resedaeformis*, *Pa Paragorgia arborea*, *Bs Bathypates* spp., and *Ss Sea pens*. *TN* tree node. All data are expressed as mean percentage ± standard deviation

species show an average of 10.3% for depths less than 876 m, an increase to 13.6% for depths between 876 and 1,157 m and a subsequent decrease for depths >1,157 m (7.7%).

When establishing a decision tree with only *Depth* as a predictor variable without considering differences in species, this trend appeared more clearly with an increase between 876 and 1,200 m followed by a decrease after 1,200 m (Fig. 3b).

Differences in TG values were observed between species with low levels for Ag, Gr, Aa, Ps, and Pr, and higher levels for Pa, antipatharians, sea pens, and Cf (Fig. 1d; Table 2). These differences did not seem to be associated with species belonging to a particular group of corals. When establishing a decision tree with only *Depth* a trend could be seen with an increase between 959 and 1,124 m from 6.6 to 16.3% and a subsequent decrease after 1,124 m ($n = 6$ samples at lower depths) (Fig. 3c).

DTA carried out with all three predictors for MADAG showed a decrease of percentages at depths greater than 150 m, as well as differences between species with Ag, Gr, Aa, Ps, and Pr, Pa and sea pens containing lower levels than antipatharians, and Cf (Fig. 4a; Table 2). The same threshold (150 m) could be seen when applying only one predictor variable to the decision tree analysis. It is important to note that most of the eight specimens sampled at depths less than 150 m were Gr and Cf rendering conclusions on an actual depth trend difficult (Fig. 3d).

When applying DTA with three predictor variables for WAX, results showed no dependency on depth. Moreover, differences between species showed higher levels of WAX in antipatharians, Cf, Pr, Gr, and sea pens (Fig. 4b; Table 2).

When applying all predictors to SE decision tree, a depth trend was observed only for Pa, antipatharians, Ps, and Pr (Fig. 4c; Table 2). This trend appeared for all species when applying only *Depth* as a predictor variable to the DTA, with SE values increasing after 828 m (Fig. 3e).

The observations gathered after application of the classification trees were used to visualize a depth trend using all data (Fig. 5). The decrease in percentage observed after 1,200 m for STEROLS was significant (t test, $P < 0.05$). Similarly, SE values increased significantly after 828 m (t test, $P < 0.05$). The increase of percentages observed

Table 2 One-way ANOVA and multiple comparison procedures (Holm-Sidak) applied to tree nodes after DTA (three predictor variables)

Data	ANOVA p value	Multiple comparisons	Significance
Total lipids	$P < 0.001$	Tree node 1 vs. node 2	Yes
		Tree node 1 vs. node 3	Yes
		Tree node 1 vs. node 4	Yes
		Tree node 2 vs. node 3	No
		Tree node 2 vs. node 4	Yes
		Tree node 3 vs. node 4	No
STEROLS	$P = 0.005$	Tree node 1 vs. node 2	Yes
FFA	$P < 0.001$	Tree node 1 vs. node 2	No
		Tree node 1 vs. node 3	Yes
		Tree node 1 vs. node 4	Yes
		Tree node 2 vs. node 3	No
		Tree node 2 vs. node 4	Yes
		Tree node 3 vs. node 4	No
TG	$P = 0.001$	Tree node 1 vs. node 2	Yes
MADAG	$P = 0.013$	Tree node 1 vs. node 2	No
		Tree node 1 vs. node 3	No
		Tree node 2 vs. node 3	Yes
WAX	$P = 0.028$	Tree node 1 vs. node 2	Yes
SE	$P = 0.008$	Tree node 1 vs. node 2	Yes
		Tree node 1 vs. node 3	Yes
		Tree node 2 vs. node 3	No

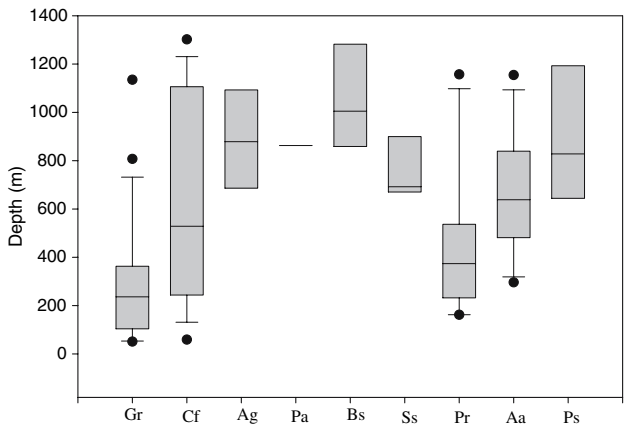

Fig. 2 Depth distribution of coral samples. *Ag Anthomastus grandiflorus*, *Gr Gersemia rubiformis*, *Cf Capnella florida*, *Aa Acanella arbuscula*, *Ps Paramuricea* spp., *Pr Primnoa resedaeformis*, *Pa Paragorgia arborea*, *Bs Bathypates* spp., and *Ss Sea pens*. The ends of the *boxes* define the 25th and 75th percentiles, with a line at the median, and *error bars* defining the 10th and 90th percentiles. Data for *Pa* does not appear on the graph because $n = 3$ (depth ranges can be found in Table 1)

after 959 or 876 m was significant for TG but not for FFA, respectively, while the subsequent decrease at depths greater than 1,200 and 1,124 m was significant for FFA but not TG (Table 3).

Discussion

The technique used for lipid extraction resulted in a coefficient of variation of 10%. Harland et al. (1993) found between 5 and 10% variation in lipid percentages between samples from the same colony (CV of 6–17%), confirming the importance of replicating lipid extraction. Lipid percentages of deep-water corals were lower than values obtained for their shallow-water counterparts. Values ranged from 2.4 to 38.8% while lipid percentages found in shallow-water species varied between 6 and 47% (Harland et al. 1993; Grottoli et al. 2004). Shallow-water corals are essentially photoautrophic with respect to carbon, have high rates of growth compared to deep-water corals (Huston 1985) and are therefore able to maintain high lipid levels (Stimson 1987). Decision-tree analysis revealed a link between coral groups and differences in total lipid percentages showing that species within the same group had similar lipid amounts. The following sequence was found: total lipids in gorgonians < soft corals and sea pens < order antipatharia.

MADAG and WAX were the most represented lipid classes in the coral samples investigated. Conversely, the only other study on lipids in deep-sea coral species known to the authors conducted by Kiriakoulakis et al. (2005), showed a domination of fatty acids in *Lophelia pertusa* and *Madrepora oculata*. On the other hand, most shallow water corals have been shown to have high levels of WAX and TG (Yamashiro et al. 1999, 2005). Two species sampled in Hawaii had high phospholipid percentages (Grottoli et al. 2004), thus highlighting the complexity of the distribution of lipid classes in corals.

Wax esters and triacylglycerols are the main storage lipids in shallow corals and can account for 40–73% of total lipids (Harland et al. 1993; Yamashiro et al. 1999). Alkyldiacylglycerols (like MADAG) can be metabolized to yield energy but at a much lower rate than triacylglycerols (Sargent et al. 1973). The study of lipoproteins of sharks (Sargent et al. 1973; Mills et al. 1977) as well as some primitive coelacanth (Mills and Taylaur 1973) showed that MADAG is a feature of "primitive" lipid metabolism which has been lost during the evolution of teleosts and higher classes of animals (Mills et al. 1977). The results of the present investigation suggest that the deep-sea corals considered here are storing energy mostly as MADAG (14.4%) and WAX (14.0%), In fact, Giese (1966) suggests that lipid in excess of 5% of invertebrate dry body weight is used as an energy reserve. The TG percentages of most coral species studied were among the lowest (7.71%) of all classes considered, suggesting that MADAG (alkyldiacylglycerols) is used preferably to TG as an energy reserve.

Unidentified grey areas were observed between FFA and TG on some HPTLC plates (29 plates of the 70 plates

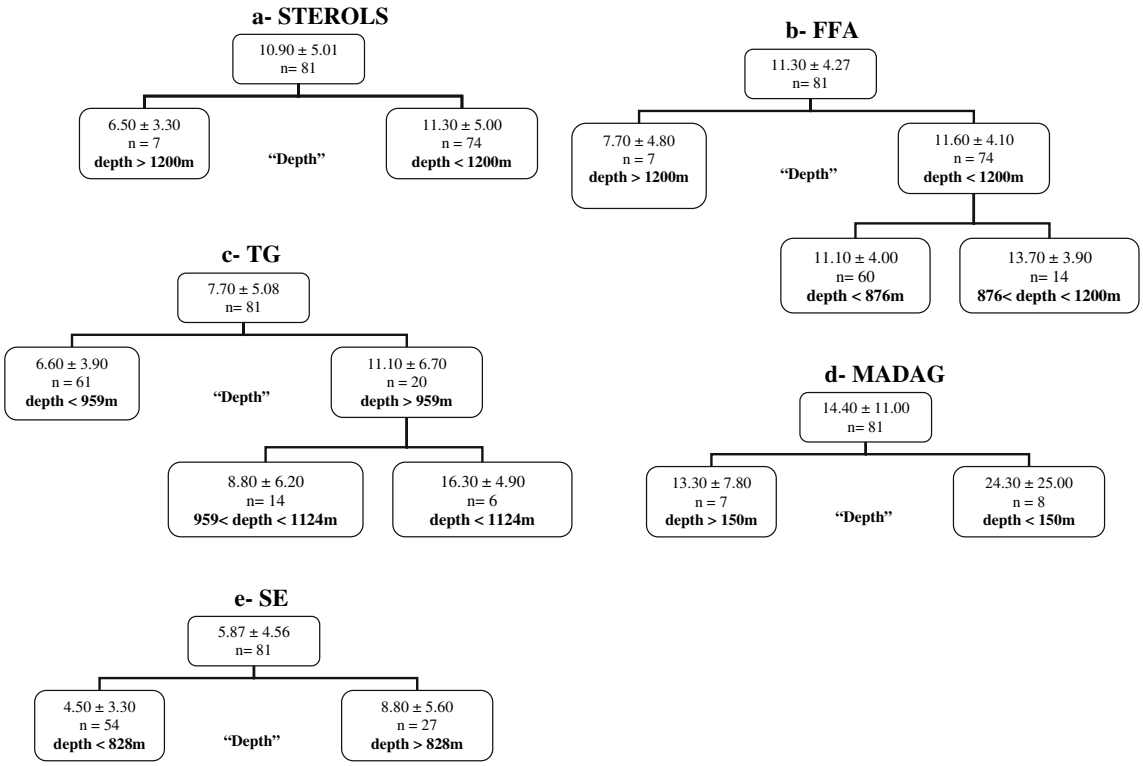

Fig. 3 Decision tree analysis for **a** *STEROLS*, **b** *FFA*, **c** *TG*, **d** *MADAG*, and **e** *SE* considering only the *Depth* variable. All data are expressed as mean percentage ± standard deviation

analyzed). Oku et al. (2002) noted the presence of unknown lipids in the same area of the plate and found them to be associated with a single genus (*Montipora*). In this study, these unknown lipids were found in some of the samples only; further studies are necessary to identify these lipids and establish an eventual association with particular species and/or genus.

In this investigation, data was combined from different species with different requirements in terms of depth and/or temperature. Decision tree analysis was applied to explore lipid distributions and depth trends. DTA showed that the variable *Species* was important in explaining differences in lipids. In most cases, differences between lipid percentages (total lipids and lipid classes) within species went beyond disparities only due to discrepancies in the depth distribution of species. Nonetheless, it is important to note that decision trees suffer from two drawbacks: instability and masking (Breiman et al. 1984). Masked variables may not show in the decision tree thereby hindering the understanding of the results (Hautaniemi et al. 2005). Unbalanced data and complex interaction between *Species* and *Depth* can contribute to the masking of some depth effects. Differences in lipid data due to *Species* could be therefore also associated with the effect of *Depth*. The impact that *Depth* has on lipid composition was emphasized in this manuscript for the reason that changes in lipids associated with depth variation (i.e., temperature, flow, and nutrients) bring an insight to the biology of corals in relation to their environment. Many factors that co-vary with depth can be expected to determine vertical distribution patterns of corals (Mortensen and Buhl-Mortensen 2004). In the North-east Atlantic different regional maximum depths of deep-water corals in offshore areas generally reflect different maximum depths of water with suitable temperatures (Mortensen et al. 1995; Freiwald and Roberts 2005). Most of the groups identified after DTA application were significantly different (ANOVA). In the DTA used in this study, splitting is performed by selecting the predictor resulting in the smallest within-group sum-of-squares for a binary split. The objective is to partition data into homogeneous groups, but also keep the tree reasonably small (De'ath and Fabricius 2000). These groups, even when not significantly different, bring important information as they reveal trends in often complex and unbalanced ecological data.

Depth trends were seen for FFA, TG and SE with an increase after 800 m and for FFA and TG a subsequent decrease after 1,000 m. The increase observed in FFA, TG and SE after 800 m could suggest a need for storage because of a decrease in food availability. The decrease in some lipids at depths greater than 1,000 m warrants a more detailed investigation as the number of specimens acquired from these depths was less than 20. It is nonetheless a

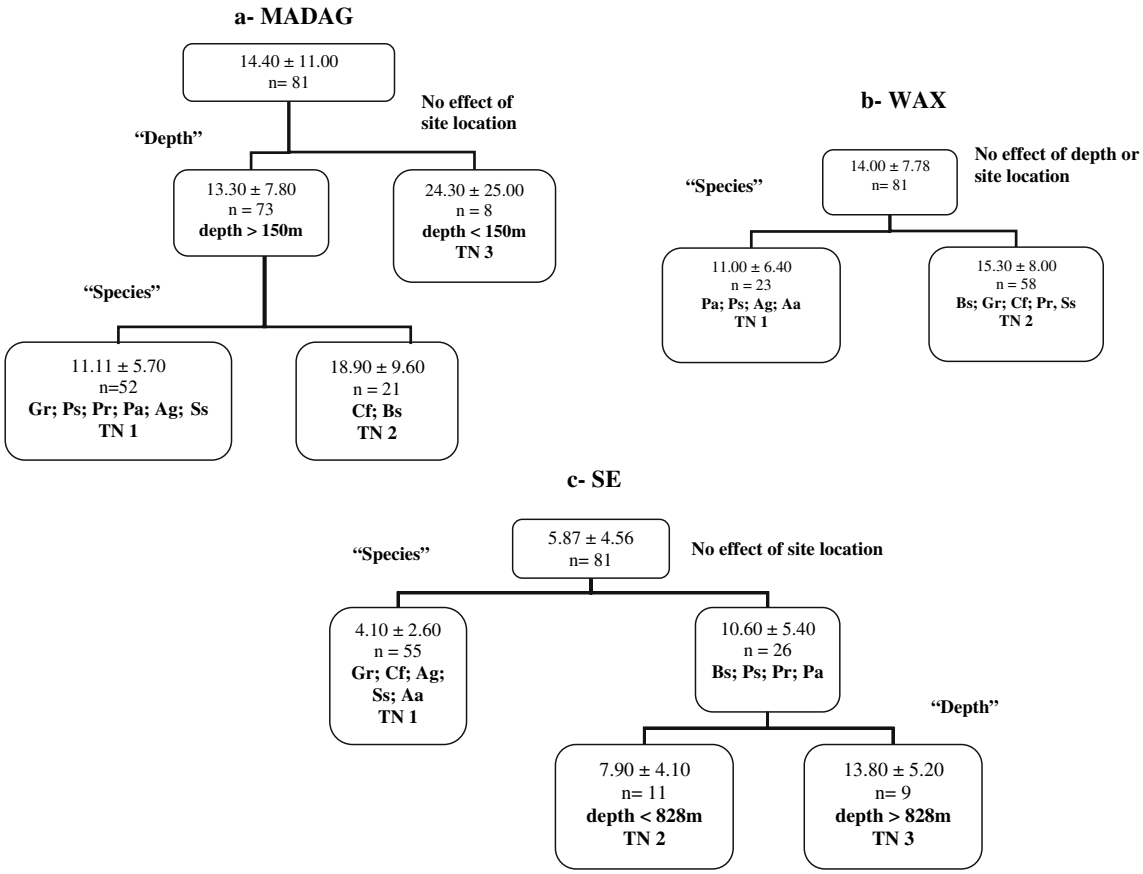

Fig. 4 Decision tree analysis for **a** *MADAG*, **b** *WAX*, and **c** *SE* with three variables: *Depth*, *Species* and *Location*. *Ag Anthomastus grandiflorus*, *Gr Gersemia rubiformis*, *Cf Capnella florida*, *Aa Acanella arbuscula*, *Ps Paramuricea* spp., *Pr Primnoa resedaeformis*, *Pa Paragorgia arborea*, *Bs Bathypates* spp., and *Ss Sea pens*. *TN* tree node. All data are expressed as mean percentage ± standard deviation

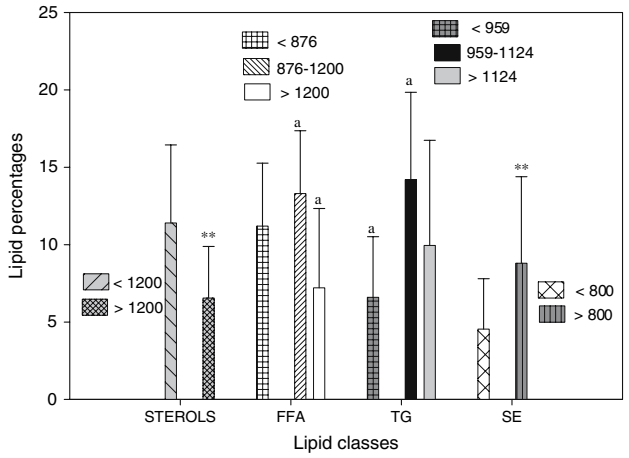

Fig. 5 Depth profiles of three classes of lipids (all species are included). *Asterisk* significant statistical difference when compared to values <800 m, or <1,200 m (*t* test, $P < 0.05$). *a* groups with significant differences after application of Holm-Sidak multiple comparison procedure

surprising result as increased storage is to be expected when food sources are sparse. Analysis of particulate organic matter reaching the deep-sea floor shows a high percentage of fatty acids and sterols (Kiriakoulakis et al. 2004). Sterols may be present in a free form or sterified with fatty acids. Free sterols form part of membranes and play an important role in regulating their viscosity and permeability (Nes 1974). In mollusks, a decrease in sterols has been observed following spawning suggesting that sterols are required for forming new cellular membranes during gamete proliferation (Pollero et al. 1983; De la Parra et al. 2005). The decrease in sterols observed below 1,000 m could result in reduced access to sterol with increasing depths. This, in turn, could be associated with low growth rate of corals (Watling and Norse 1998) and increased longevity (Sherwood et al. 2005) resulting in decreased requirements in structural lipids. Similarly, the increased levels of FFA, TG, and SE observed after 800 m could also be related to decreased cell proliferation. Oku et al. (2002) showed that decreased storage lipid (TG and wax) in a shallow-water species *Montipora digitata* was associated with the increased energy expended by the proliferating cells. Effect of bleaching and stress on shallow corals has shown that corals have two strategies for lipid use: (1) to use available lipid stores, thereby depleting total percentages, or (2)

Table 3 All Pairwise multiple comparison procedure (Holm-Sidak) applied to FFA and TG percentages after ANOVA

Comparison	Unadjusted *P*	Critical value	Significance
FFA < 876 m vs. FFA 876–1200 m	0.117	0.05	No
FFA 876–1200 m vs. FFA > 1200 m	0.00428	0.017	Yes
FFA < 876 m vs. FFA > 1200 m	0.0260	0.025	No
TG < 959 m vs. TG 959–1124 m	0.0000265	0.017	Yes
TG 959–1124 m vs. TG > 1124 m	0.0504	0.05	No
TG < 959 m vs TG > 1124 m	0.0334	0.025	No

to shift the lipid class composition (Grottoli et al. 2004). The fact that depth (changes in particulate organic matter, flow, temperature) did not seem to impact the total lipid percentages could suggest that deep-sea corals adapt to differential access to food by changing the proportion of lipid classes but maintaining equivalent lipid levels.

The results of this study show that deep-sea corals have fewer lipids than their shallow water counterparts. Lipids are stored as neutral lipids with a high proportion of MADAG, a compound less rich in energy than triacylglycerols. Depth trends were observed for FFA, TG and SE suggesting different metabolic rates with increasing depths as well as different survival strategies below 800 and 1,000 m. Future analysis should include fatty acid composition to bring more information on the diets of deep-sea corals. Fatty acids have been extensively studied in zooplankton ecological studies to determine trophic relationships (e.g., Falk-Petersen et al. 2000; Stevens et al. 2004). Sherwood et al. (2005) indicate that two deep-water species *Primnoa resedaeformis* and *Primnoa willeyi* feed mainly on zooplankton and/or sinking particulate organic matter. Information provided by Kiriakoulakis et al. (2005) on *Lophelia pertusa* and *Madrepora oculata* diet show that it mostly consisted of mesozooplankton.

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